DISSERTATION ON

CD44(CELL ADHESION MOLECULE) EXPRESSION IN GASTRIC ADENOCARCINOMA- PROGNOSTIC IMPORTANCE

A STUDY OF 50 CASES

Dissertation submitted to

Tamil Nadu Dr. M.G.R. Medical University

Chennai

for

M.D. (PATHOLOGY)

April 2013

Under the guidance of

Dr .R. Padmavathi, M.D.,

Professor of Pathology,

Department of Pathology

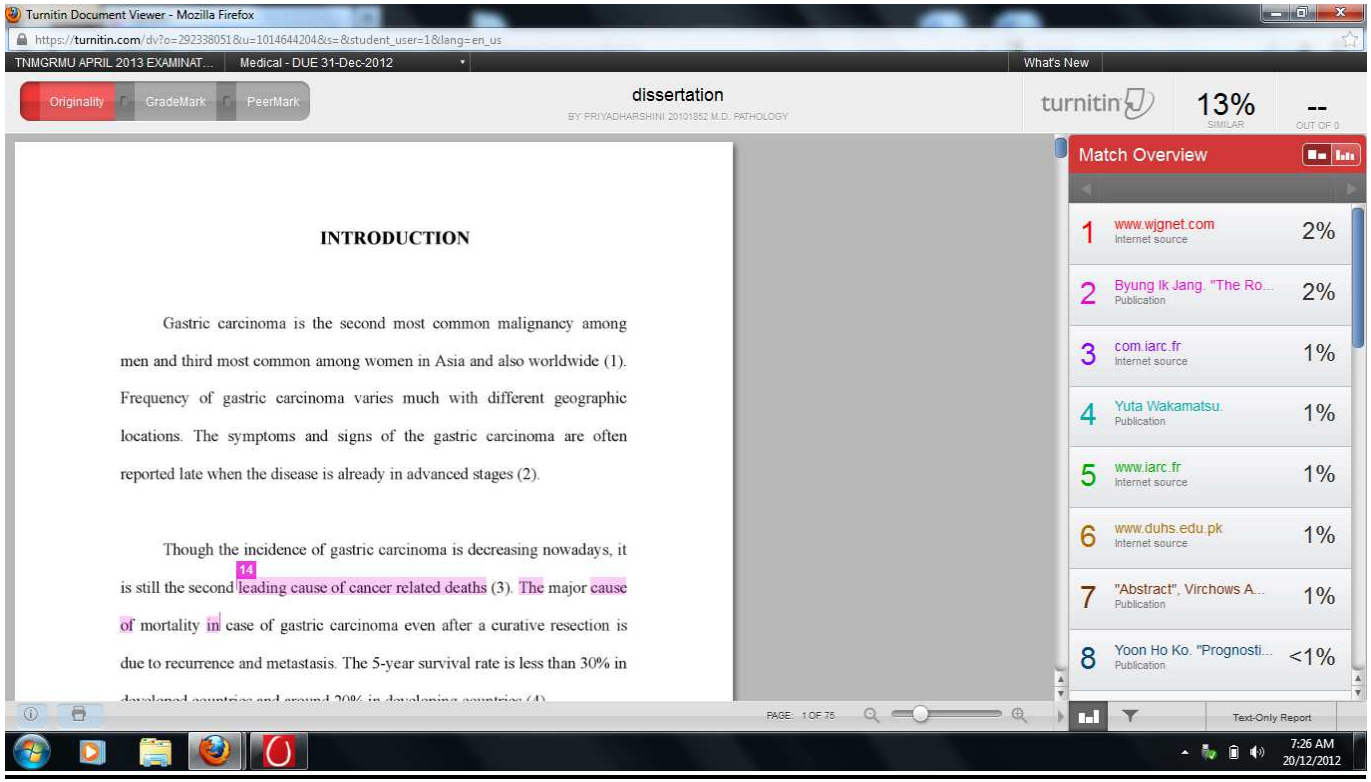
Govt .Stanley Medical College

Chennai.

THE TAMIL NADU Dr. M.G.R. MEDICAL UNIVERSITY

CHENNAI-TAMILNADU

PLAGIARISM


Turnitin Document Viewer - Mozilla Firefox
https://turnitin.com/dv?o=292338051&u=1014644204&s=&student_user=1&lang=en_us
TNMGRMU APRIL 2013 EXAMINAT...
Medical - DUE 31-Dec-2012
What's New
Originality
GradeMark
PeerMark
dissertation
BY PRIYADHARSHINI 20101852 M.D. PATHOLOGY
turnitin
13%
SIMILAR
--
OUT OF 0
INTRODUCTION
Gastric carcinoma is the second most common malignancy among men and third most common among women in Asia and also worldwide (1). Frequency of gastric carcinoma varies much with different geographic locations. The symptoms and signs of the gastric carcinoma are often reported late when the disease is already in advanced stages (2).
Though the incidence of gastric carcinoma is decreasing nowadays, it is still the second leading cause of cancer related deaths (3). The major cause of mortality in case of gastric carcinoma even after a curative resection is due to recurrence and metastasis. The 5-year survival rate is less than 30% in
Match Overview
1 www.wjgnet.com Internet source 2%
2 Byung Ik Jang. "The Ro... Publication 2%
3 com.iarc.fr Internet source 1%
4 Yuta Wakamatsu. Publication 1%
5 www.iarc.fr Internet source 1%
6 www.duhs.edu.pk Internet source 1%
7 "Abstract", Virchows A... Publication 1%
8 Yoon Ho Ko. "Prognosti... Publication <1%
PAGE: 1 OF 75
Text-Only Report
7:26 AM
20/12/2012

ACKNOWLEDGEMENTS

I would like to begin by expressing my humble gratitude to **The Almighty** for his blessings and all the good things he showered on me.

I take this opportunity to express my heartfelt gratitude to **Dr. S. Mary Lilly, M.D.**, Professor and Head of the Department of Pathology, Stanley Medical College, Chennai for her keen interest, constant encouragement, guidance and valuable suggestions throughout this study.

I would like to express my sincere gratitude for my guide, **Dr.R.Padmavathi, M.D.,** Professor of Pathology, Govt .Stanley Medical College for her support to me to think independently, the freedom to make mistakes and the guidance to keep me on track. I am extremely grateful to her.

I am extremely thankful to **Dr. V. Ramamoorthy, M.D.,** Professor of Pathology, Stanley Medical College who has extended his support and encouragement during the study.

My sincere thanks to **Dr. P. Arunalatha, M.D.,** Professor of Pathology, Stanley Medical College for her immense help and valuable suggestions and also for her support to perform this study.

I owe my humble thanks to **Dr. Nalli. R. Sumithra Devi, M.D.,** Professor of Pathology, Stanley Medical College who inspired, and motivated me through my study even during stressful times.

I also thank **Dr.K. Chandramouleswari**, Associate Professor of Pathology, Stanley Medical College, for her support and encouragement during the study.

It gives me immense pleasure to thank my co-guide **Dr. R. Sathyalakshmi, M.D.,** Assistant Professor, Department of Pathology, Stanley medical college who has extended her valuable guidance and support during the study.

I express my sincere thanks to **Dr. S. Jagannathan, M.D., DPH,** Assistant professor, Department of Pathology, Kilpauk Medical College who has extended his guidance and valuable suggestions for statistical analysis in the study.

I am grateful to all the faculty members, my colleagues and my loving family members for their constant support and encouragement during the period of study.

I preserved the last for the best, who are my lab technical staff persons and of course my dear friends and also all the patients, who made this study possible.

CERTIFICATE

This is to certify that this dissertation titled "***CD44(CELL ADHESION MOLECULE) EXPRESSION IN GASTRIC ADENOCARCINOMA-PROGNOSTIC IMPORTANCE. A STUDY OF 50 CASES***" is the original and bonafide work done by **Dr. J. Priyadharisini** under the guidance of Dr. R. Padmavathi, M.D., Professor, Department of Pathology at the Government Stanley Medical College & Hospital, Chennai – 600 001, during the tenure of her course in M.D. Pathology from May-2010 to April-2013 held under the regulation of the Tamilnadu Dr. M.G.R. Medical University, Guindy, Chennai – 600 032.

PROF.S.MARY LILLY,M.D.
PROFESSOR AND HEAD
Department of Pathology
Govt. Stanley Medical College
Chennai- 600 001

Place : Chennai
Date : .12.2012

PROF.S.GEETHALAKSMI,M.D,PhD
DEAN
Govt. Stanley Medical College & Hospital
Chennai-600 001

Place : Chennai
Date : .12.2012

CERTIFICATE BY THE GUIDE

This is to certify that this dissertation titled "***CD44(CELL ADHESION MOLECULE) EXPRESSION IN GASTRIC ADENOCARCINOMA-PROGNOSTIC IMPORTANCE. A STUDY OF 50 CASES***" is the original and bonafide work done by **Dr. J. Priyadharisini** under my guidance and supervision at the Government Stanley Medical College & Hospital, Chennai – 600 001, during the tenure of her course in M.D. Pathology from May-2010 to April-2013 held under the regulation of the Tamilnadu Dr. M.G.R. Medical University, Guindy, Chennai – 600 032.

PROF. R. PADMAVATHI, M.D.,
Professor
Department of Pathology
Government Stanley Medical College
Chennai- 600 001.

Place : Chennai
Date: .12.2012

DECLARATION BY THE CANDIDATE

I solemnly declare that this dissertation titled "***CD44 (CELL ADHESION MOLECULE) EXPRESSION IN GASTRIC ADENOCARCINOMA-PROGNOSTIC IMPORTANCE. A STUDY OF 50 CASES***" is the original and bonafide work done by me under the guidance of Dr. R. Padmavathi, M.D., Professor, Department of Pathology at the Government Stanley Medical College & Hospital, Chennai – 600 001, during the tenure of my course in M.D. Pathology from May-2010 to April-2013 held under the regulation of the Tamilnadu Dr. M.G.R. Medical University, Guindy, Chennai – 600 032.

Place : Chennai **Signature by the candidate**
Date: .12.2012 **Dr. J. Priyadharisini**

CONTENTS

ABBREVIATIONS

APC	**-**	**Adenomatous polyposis coli.**
CD44	**-**	**Cluster of differentiation 44.**
D2	**-**	**Second part of duodenum.**
HA	**-**	**Hyaluronic acid.**
HRP	**-**	**Horse radish peroxidase.**
IARC	**-**	**International Agency For Research On Cancer.**
IGFR II	**-**	**Insulin like growth factor receptor 2**
IHC	**-**	**Immunohistochemistry.**
LP	**-**	**Lamina propria**
MD	**-**	**Moderately differentiated adenocarcinoma.**
PBCR	**-**	**Population Based Cancer Registries.**
PD	**-**	**Poorly differentiated adenocarcinoma.**
RT-PCR	**-**	**Real time polymerase chain reaction**
TGFβR II	**-**	**Transforming growth factor receptor 2**
TNM	**-**	**Tumor Node And Metastasis.**
WD	**-**	**Well differentiated adenocarcinoma.**

INTRODUCTION

INTRODUCTION

Gastric carcinoma is the second most common malignancy among men and third most common among women in Asia and also worldwide (1). Frequency of gastric carcinoma varies much with different geographic locations. The symptoms and signs of the gastric carcinoma are often reported late when the disease is already in advanced stages (2).

Though the incidence of gastric carcinoma is decreasing nowadays, it is still the second leading cause of cancer related deaths (3). The major causes of mortality in case of gastric carcinoma even after a curative resection are recurrence and metastasis. The 5-year survival rate is less than 30% in developed countries and around 20% in developing countries (4).

Gastric adenocarcinoma comprises 95% of the total malignancies of stomach. Multiple genetic and epigenetic alterations are known to involve oncogenes, tumor suppressor genes, cell cycle regulators, cell adhesion molecules and are implicated in the multistep carcinogenesis of human gastric carcinoma(8).

HISTOLOGICAL AND ANATOMICAL CLASSIFICATION OF GASTRIC CANCER:

Several classification systems have been proposed to delineate gastric cancer on the basis of both macroscopic as well as microscopic patterns of growth. Different classification system includes Borrman, Stout, Japanese system, Ming, World Health Organization (WHO) system and Lauren's method. Of which WHO and Laurens are the two most commonly used classification systems (5).

Treatment of choice for any resectable gastric cancer is, surgical therapy which could be total gastrectomy, subtotal gastrectomy or proximal gastrectomy. The choice of procedure depends on the location of tumor and the ability to achieve tumor free surgical margins.

The current TNM staging classified nodal metastasis based on number of positive nodes, in which it advocates a minimum of 15 lymph nodes to be resected and examined for staging to be accurate. Here comes the extended D2 resection procedure which offers better survival advantage compared with other surgical resections without lymphadenectomy(7).

Though with the availability of advanced methods of surgical techniques, it is still common to see patients with recurrence in many cases.

Despite knowing that gastric carcinoma is not very sensitive to current chemotherapy agents, most of them are considered as palliative in order to reduce the tumor burden, to provide symptomatic relief and for better survival time. Post operative adjuvant chemotherapy can to some extent decrease the rate of recurrence.

Though we know that gastric carcinoma is prone for recurrence, even with curative resection and modern chemotherapeutic drugs, no molecular biomarker is currently available to predict gastric carcinoma recurrence before / after resection. Frequent cause of death in gastric carcinoma are recurrence and metastasis.

Metastasis is initiated primarily by the loss of adhesion that renders the cancer cells to leave the site of origin, and subsequently reach the distant sites such as lymph nodes, liver, or the peritoneum to form secondary cell colonies (9).

Interaction between the cells and also between the cell and the extracellular matrix is strictly regulated by cell adhesion molecules. Any

breakthrough in this property of cell adhesion molecules allow the process of tumor progression and metastasis(10).

CD44 is a principal cell surface receptor for hyaluronic acid. Hyaluronic acid is a major component of extracellular matrix. Increased expression of CD44 by the tumor cells leads to avid binding with the hyaluronan in the matrix, thereby helping in communication of cell-matrix interactions into the cell which is known as outside-in signalling(10).

CD44 plays a role in carcinogenesis, differentiation, as well as lymph node metastasis which are considered to be prognostic for carcinomas of various organs such as lung , breast , pancreas, gastrointestinal tract including gastric cancer. It is considered as a determinant of metastatic and invasive behavior(11).

As the literature suggests, CD44 is one of the factor that shows better association with initiation and progression of gastric cancer and also plays major role in diagnosis, therapy as well as in prognosis. The objective of this study is to identify the role of an adhesion protein CD44 in the genesis of gastric carcinoma.

The primary techniques that help to evaluate the presence and degree of CD44 expression are immunohistochemistry, fluorescence cell sorting, and reverse transcriptase polymerase chain reactions.

Research on CD44 has been active but fragmented, and it may offer newer therapeutic approach to gastric cancer.

While clinical predictive factors, such as tumor staging, can predict recurrence of advanced gastric cancer, some group of molecular-based biomarkers like CD44 which is a cell adhesion molecule can serve as a useful predictor for recurrence of advanced gastric cancer after curative resection and it has some possible predictive relevance in future clinical practice.

AIMS AND OBJECTIVES

AIMS AND OBJECTIVES:

1. To analyse age and sex distribution of gastric adenocarcinoma
2. To analyse site of distribution of gastric adenocarcinoma.
3. To assess the level of CD44 expression in gastric adenocarcinoma
4. To evaluate the relation of cluster of differentiation 44 (CD44) expression with the pathological features like histological type, grade and stage of gastric adenocarcinoma.

REVIEW OF LITERATURE

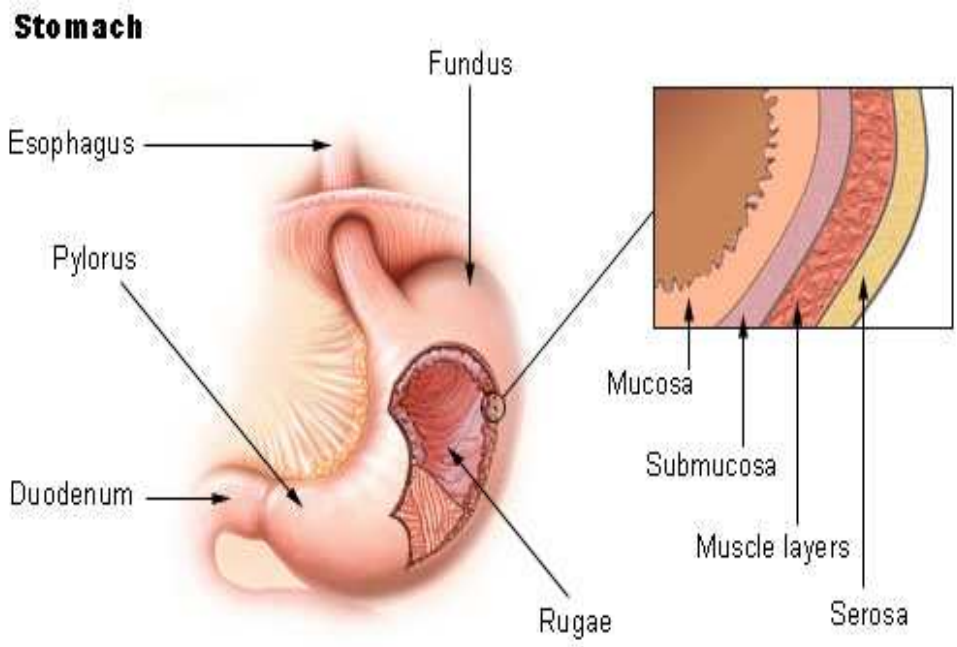
REVIEW OF LITERATURE

Gastric carcinoma ranks second for cancer related mortality worldwide.

HISTORICAL REVIEW:

The word stomach is derived from the Latin name called ‘stomachus’. The stomach is a hollow muscular dilated region of the digestive system. It lies between the esophagus and the small intestine.

The stomach lies below the diaphragm. The major part of stomach bed is formed by the pancreas. The greater curvature of the stomach is surrounded by the greater omentum. There are two sphincters which keep the contents of the stomach contained, esophageal sphincter dividing the digestive tract above, and the pyloric sphincter which divides the stomach from the small intestine.



STOMACH

- Anatomically it is divided into 4 regions. They are cardia, fundus, body & antropyloric region.
- Histologically the stomach is divided into 3 regions, which includes cardia, body and pylorus, because the fundus & the body have identical microscopic features.

Layers of the stomach wall includes mucosa, submucosa, muscularis externa and serosa.

HISTOLOGY OF STOMACH

MUCOSA

Epithelium: Simple columnar lining epithelium that invaginates to variable extent into underlying lamina propria forming gastric pits.

Lamina propria:

Composed of loose connective tissue, and it is packed with glands.

Muscularis mucosa: Smooth muscle fibers.

SUBMUCOSA

Made of dense connective tissue, blood and lymph vessels as well as submucosal plexus of nerves, but absent glands.

MUSCULARIS EXTERNA

Characterized by 3 layers of smooth muscle which are inner-oblique, middle-circular & outer-longitudinal. In between the middle and outer muscular layers, lies myenteric plexus which innervates the wall.

SEROSA

Thin layer of connective tissue & mesothelium.

GASTRIC GLANDS are of 3 types based on the distribution and structural differences. They are cardia glands, fundic glands and pyloric glands. Each gland has 3 regions such as neck, isthmus (body) & base. Five types of cells which includes **stem cells**, mucus neck cells, parietal cells, chief cells and entero-endocrine cells are found in these glands.

EPIDEMIOLOGY

According to the World Health Organization, gastric carcinoma is the fourth most common malignancy worldwide, with gastric cancer cases accounting for the burden of around 8,70,000 new cases every year(16). Almost two-thirds of the cases occur in developing countries and 42% in China alone.

The rate of occurrence of gastric cancer in different parts of world is as follows:

INDIA -8.9/100000

USA -10/100000

JAPAN -79.9/100000

ENGLAND -18.5/100000.

This wide difference in incidence of gastric carcinoma in various geographic locations can be attributed to environmental factors and also due to differences in lifestyle and dietary habits. This property leads to division of high and low incidence areas for gastric carcinoma. Areas showing higher incidence includes East Asia (China, Japan) which accounts for major case percentage of 42% followed by Eastern Europe and also parts of Central and South America(18).

Incidence rate in Chennai was 232 cases per 100,000 population and age standardised rate is around 12.3 and is more prevalent in age group of more than 60 years of age(18). Gastric cancer ranks first among males, but third among female cancers according to Chennai population based cancer registries(20).

The age adjusted incidence rates for stomach cancer was found to be 16.4 per 100000 male and 6.5 per 100000 female population based on Chennai PBCR(23).

Despite newer surgical techniques and better chemotherapeutic agents, 40% of patients with advanced gastric cancer die of recurrence (17). The prognosis for patients after curative surgery still remains poor due to the

high recurrence rate. The 5-year survival rate for patients with post-resection status for gastric carcinoma range from 47% to 60.4%.

CLINICAL MANIFESTATIONS

The symptoms are often non-specific in early stages of gastric carcinoma, due to which there is often delay in the diagnosis. Most common symptoms are anorexia, weight loss and abdominal pain.

By the time, the signs and symptoms appear, the stage of gastric cancer would be advanced. Nausea, vomiting and early satiety occurs more commonly with bulky tumors that cause obstruction or infiltrative tumors that affects the normal distending capacity of the stomach. Ulcerated tumors may cause bleeding which may lead to hematemesis or malena(27).

RISK FACTORS:

Environmental factors play a major role in the risk stratification among individuals. The role of H.pylori infection in pathogenesis of gastric carcinoma is well established by many studies. It is considered as carcinogenic in humans, as it is suggested by IARC.

High prevalence of gastric carcinoma within the population of lower socioeconomic status is certainly related to increased occurrence of H.Pylori infection in these people.

The Cag A gene (49) is the main virulence factor of H. pylori which is responsible for the development of gastric adenocarcinoma through the derangement of cellular architecture. The studies conducted in India showed high prevalence of H.Pylori which varies from 56-89% among cases of gastric carcinoma(4) and it also showed major association with lower socioeconomic status.

Diet plays a major role in gastric carcinogenesis. Globally, literature suggests that none or low-starch vegetable is considered, the probable protective factor. In western diets where the animal foods and food preservatives are commonly used, there is increased risk of gastric carcinoma(21).

Tobacco use in any form (chewing, smoking and drinking) was observed to increase the risk of stomach cancer in India. Smoking tobacco has been found to be an independent risk factor for stomach cancer.

Literature suggests that alcohol may be carcinogenic to the esophagus and cardia cancers but not to distal gastric cancer(22).

MORPHOLOGY OF GASTRIC CARCINOMA

Gross appearance of gastric cancer varies and can be either polypoidal, fungating, ulcerative or scirrhous.

Polypoidal and fungating type of tumors tend to produce intraluminal mass. Polypoid tumors generally are not ulcerated; but the fungating tumors are ulcerated.

In the other two gross forms of tumor such as ulcerative and scirrhous type, the bulk of the tumor mass is in the wall of the stomach.

Scirrhous tumors which are otherwise called as “Linitis Plastica” tend to infiltrate the entire thickness of wall of the stomach leading to leather bottle appearance. It has particularly poor prognosis, and commonly involve the entire stomach.

CLASSIFICATIONS OF GASTRIC CARCINOMA

Many classification systems of gastric carcinoma has been proposed based on tumor location, invasiveness, degree of differentiation, growth pattern and histological features.

Based on **tumor location** it is divided into **cardia and non-cardia** cancers of stomach. These two anatomical subtypes show remarkable epidemiological and etiological differences.

Non-cardia cancer is the predominant type seen in high risk areas and generally thought to arise due to the interaction between environment, host and H. pylori factors. But the cardia gastric cancer is more homogenously distributed throughout the world and develops by two major etiological mechanisms -atrophic gastritis and intestinal metaplasia. Though the overall incidence is declining, the cancer of gastric cardia is on the rise.

Based on **invasiveness**, it is classified into two types as **early gastric cancer and late gastric cancer**.

In early type, invasion is restricted to submucosa, which is further subdivided into intramucosal and submucosal types. In the late type, invasion of muscularis externa is present.

Based on the **degree of differentiation**, gastric adenocarcinoma is divided into three types as **well differentiated, moderately differentiated and poorly differentiated adenocarcinoma** (classified based on the proportion of resemblance of tumor to the normal counterpart). In general the degree of gland formation is widely regarded as most important feature in grading.

In well differentiated adenocarcinoma, >95% of tumor is composed of well developed tubular glands with uniform cytological features. In moderately differentiated adenocarcinoma, complex glands with cribriform pattern is seen in around 50-95% of tumor cell population. In poorly differentiated adenocarcinoma, solid nests of cells, single cells, and signet ring cells with variable cytological abnormalities are seen (<49% of tumor composed of glands).

This system is simple and reproducible but may be difficult to apply because of morphological heterogeneity within the same tumor present in many neoplasms.

Based on **the growth pattern,** gastric carcinoma is divided into two types as **expansile and infiltrative types**.

The expansile type of tumors, grow as cohesive cell groups but the infiltrative tumors tend to show a diffusely infiltrative edge.

Most important among these systems are WHO and Laurens classification system.

WHO(19) categorises the grades to adenocarinoma based on the degree of resemblance to metaplastic intestinal tissue, by which it identifies **5 major subtypes of adenocarcinoma**(24) like adenocarcinoma (intestinal, diffuse type), papillary, mucinous and signet ring cell carcinoma type.

Laurens has classified gastric carcinoma as **intestinal and diffuse types**-which shows difference in both geographic as well as histologic features(6). The morphological difference in these two types are attributed to intercellular adhesion molecules which are very well preserved in intestinal type rather than diffuse type of gastric carcinoma where there is loss of expression of E–Cadherin.

“WHO classification of tumors of stomach(19)”:

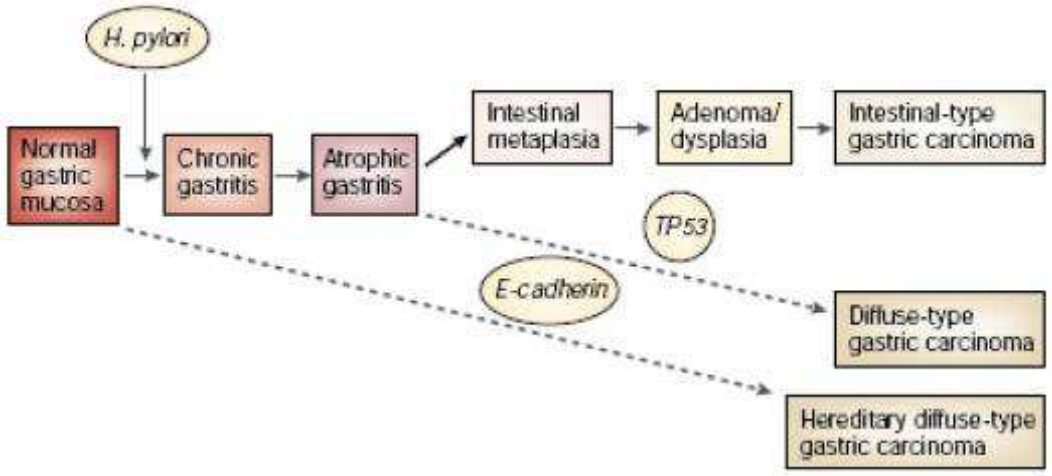
1. **Epithelial tumours**	2. **Non-epithelial tumours**
A.Intraepithelial neoplasia	Leiomyoma
	Schwannoma
B. Carcinoma which includes	Granular cell tumour
Adenocarcinoma	Glomus tumour
-intestinal type	Leiomyosarcoma
-diffuse type	Gastrointestinal stromal tumour
Papillary adenocarcinoma	-benign
Tubular adenocarcinoma	-uncertain malignant potential
Mucinous adenocarcinoma	-malignant
Signet-ring cell carcinoma	Kaposi sarcoma
Adenosquamous carcinoma	Others
Squamous cell carcinoma	**Malignant lymphomas**
Small cell carcinoma	Marginal zone B-cell lymphoma of MALT-type
Undifferentiated carcinoma	Mantle cell lymphoma
Others	Diffuse large B-cell lymphoma
C. Carcinoid	**3. Secondary tumours**

Laurens classification system:

	Intestinal	Diffuse type
Epidemiology	High risk areas only	Even in low risk areas
Environmental factors	Present – H.Pylori	Absent
Male:Female	2:1	M=F
Age of patients	>50 years	<50 years
Anatomical site	Antrum	Fundus/body
Growth pattern	Exophytic, intraluminal mass with expansile growth pattern	Ulcerative, diffuse infiltrative growth pattern
Precursor lesions (chronic atrophic gastritis, metaplasia, dysplasia)	Present	Not seen always
Microscopic features	Well defined gland formation present, mucin within gland lumina.	Poorly differentiated discohesive cells, no well defined glands, intracellular / extracellular mucin+.
Prognosis	Good	Poor

PATHOGENESIS OF GASTRIC CARCINOMA:

The pathogenesis of gastric cancer is a complex and multifactorial process, which is a classical example of gene-environment interactions. The precise mechanism underlying this is not yet fully understood and vary according to the histological type of the malignancy.



The majority of the cases of gastric carcinoma, are the end products of inflammatory cascade that progresses from superficial nonatrophic gastritis to gastric atrophy and can progress to intestinal metaplasia and precursor lesions like gastric adenoma/ dysplasia allowing to form the overt invasive carcinoma. The occurrence of this histological sequence of events to form overt gastric cancer is called as CORREA pathway.

This cascade which is activated by infection with the H.Pylori is necessary, but not sufficient enough to produce gastric cancer. Chronic gastritis stimulates the proliferation and transformation of the adult stem cells which are normally located in the isthumus of corpus glands or at the base of antral glands. Either of these transformed stem cells or the bone marrow derived stem cells which have been recruited to the gastric mucosa following chronic gastritis or both of which can contribute to tumor development.

These oncogenically transformed stem cells are called as gastric cancer stem cells(GCSCs) which has similar properties of other stem cells such as self renewal and multipotential.

The two major classes of genes that have been implicated in molecular pathogenesis of gastric carcinoma are as follows:

1. Genes that are related to the regulation of cell cycle like cyclin, p53 & cyclin dependent kinases.
2. Genes that are related to growth signal transduction like growth factors and receptor tyrosine kinase.

Germline mutation in CDH1 gene which codes for E-cadherin protein that mediates epithelial intercellular adhesion, is the key step in development of the most familial types of gastric cancer.

Mutations in β catenin, APC, microsatellite instability and hypermethylation of several genes like TGFβRII, IGFRII and p16/INK4a have been associated with sporadic type of gastric cancer(25).

TUMOR SPREAD:

Property of invasion and metastasis is considered as the "hallmark" for any malignant neoplasm. The loss of normal cellular adhesion plays significant role in human cancer invasion and metastasis.

Gastric carcinoma can spread by direct extension, lymphatic/ hematogenous route or through peritoneal dissemination. Diffuse type most often spreads to duodenum through submucosa and subserosa via submucosal lymphatics and thereby to peritoneal surface. Intestinal type most often metastatise via hematogenous route to liver.

Both histological types of gastric carcinoma such as intestinal and diffuse types have the equal incidence of lymph node metastasis which can spread to supraclavicular node which is then called as "Virchows node". If it

involves left axillary node then it is called as “Irish’s node” or when it metastatise to periumblical node, called as “Sister Mary Joseph’s Node”. Peritoneal seedling may involve the ovaries or the cul-de-sac, later being called as Blumer’s shelf (26-27).

The importance of lymphnode dissection lies in the detection of metastasis and appropriate tumor staging which helps in patients management.

Some of the studies that have been conducted to demonstrate the clinical significance of lymph node involvement, noted that the extent of lymph node involvement was found to be significant predictor of survival. It is also established that the presence of tumor emboli greatly influences the tumor recurrence as well as survival after curative resection.

TNM classification of gastric tumours(19)":

T – PRIMARY TUMOUR

TX - Primary tumour cannot be assessed

T0 - No evidence of primary tumour

Tis -Carcinoma in situ (without LP invasion)

T1 -Tumour invades lamina propria or submucosa

T2 -Tumour invades muscularis propria or subserosa

T3 -Tumour penetrates serosa without invasion of adjacent structures.

T4 -Tumour invades adjacent structures such as pancreas, liver, kidney, spleen, colon and also retroperitoneum.

N – REGIONAL LYMPH NODES

Nx- Regional lymph nodes cannot be assessed

N0- No regional lymph node metastasis

N1- Metastasis in 1 to 6 regional lymph nodes

N2- Metastasis in 7 to 15 regional lymph nodes

N3- Metastasis in more than 15 regional nodes

M – DISTANT METASTASIS

MX- Distant metastasis cannot be assessed

M0 - No distant metastasis

M1 - Distant metastasis

STAGE GROUPING

Stage 0	Tis N0 M0
Stage IA	T1 N0 M0
Stage IB	T1 N1 M0
	T2 N0 M0
Stage II	T1 N2 M0
	T2 N1 M0
	T3 N0 M0
Stage IIIA	T2 N2 M0
	T3 N1 M0
	T4 N0 M0
Stage IIIB	T3 N2 M0
StageIV	T4 N1, N2, N3 M0
	T1, T2, T3 N3 M0
	Any T Any N M1

This staging applies only to carcinoma.

RATIONALE OF SEARCH FOR A NEW MARKER TO PREDICT THE TUMOR BEHAVIOUR

Early detection of group of patients with increased chance of recurrence or mortality even after curative resection would help the clinician to delineate such patients and offer them a more stringent post therapeutic monitoring and provide them a more aggressive therapeutic approach. This is the rationale for the search of new markers in gastric adenocarcinoma. Adhesion molecules are promising candidates among vast group of prospective markers.

Cell adhesion molecule groups have been classified into 5 family of proteins which includes cadherins, integrins, members of immunoglobulin family, selectins and CD44 (31-32).

Cell adhesion molecules play an important role in neoplasms since intracellular adhesion and interaction of malignant cells with surrounding extracellular matrix are crucial steps necessary for invasion and metastasis(33).

Spreading neoplastic cells have to reduce their cohesion with primary tumor and reach the extracellular matrix actively, recognizing its component at the same time. On the other hand the tumor cells travelling within

vasculature (blood stream/ lymphatics) will mimic leukocytes in their adhesion property and adheres to the endothelial surface. Thus the spread of neoplasm requires a delicate balance between adhesion and anti adhesion (34).

From the above discussion made, it has been established that, for the **tumor invasion** to occur, the cancer cells should leave the colony of primary tumor and enter the surrounding tissue, pass through the blood vessels and reach the metastatic site. At the site of metastasis, it creates the tissue environment that resembles the primary site.

The property of migratory behaviour, that is acquired by epithelial tumor cells mimics that of mesenchymal cells. Variety of extracellular signals such as upregulation/ downregulation of certain proteins, stimulates the conversion of epithelial into mesenchymal cells. This striking epithelial plasticity has been named as **"Epithelial - mesenchymal transition"**.

But for the **establishment of metastasis** at that site, the cells has to go through an inverse process called **"Mesenchymal - epithelial transition"**. Both these epithelial mesenchymal transition and also the mesenchymal epithelial transition are normal essential mechanism during embryogenesis.

Cluster of differentiation 44(CD44) is a typical example of cell adhesion protein which undergoes erroneous structural and functional changes during malignant transformation. This transmembrane glycoprotein, functions as the principal transmembrane hyaluronate receptor (H-CAM) and has also been called as Phagocytic Glycoprotein-(pgp-1).

CD44 glycoprotein is encoded by a gene located on the short arm of chromosome 11. It exists in different isoforms like CD44s and CD44v which occurs due to alternative RNA splicing.

P53 is a tumor suppressor gene that acts as a key mediator, that protects the cell from various stress inducing stimuli. CD44 is repressed by p53 under normal circumstances, but in presence of p53 mutation as in carcinogenesis, CD44 expression is activated which is needed for growth and tumor initiating ability(41).

CD44v interacts with glutamate cysteine transporter in gastric mucosa thereby regulating the reduced glutathione (GSH) synthesis. This reduced glutathione acts as an antioxidant to counteract the effect of reactive oxygen species(ROS) that are produced during chronic gastritis. Thereby high CD44 in gastric cancer cells increases the protection against ROS and promotes the tumor growth(40).

The major form of CD44 in epithelial cells is CD44s(standard) which consists of extracellular domains like exons 1-5 and 16, a transmembrane domain(exon 18) and also a cytoplasmic domain (exon 20). CD44v(variant) are isoforms that are formed rather by the alternative splicing of exons 6-15(14).

The property of post translational modification of the cytoplasmic domain of CD44 by protein kinase C allows it to attach to membrane cytoskeleton called ankyrin, which enables the adhesion property (42).

PHYSIOLOGICAL FUNCTION OF CD44

CD44 was initially described as an antigen on red blood cells and platelets, which is now being termed as a lymphocyte homing receptor (28). Both the metastasizing tumor cells and recirculating lymphocytes share similar properties like motility and invasive behaviour, which indicates that tumor cells might use molecules like CD44 for metastasizing(18).

'THE CHARACTERISTIC' functions of CD44 includes both **the cell adhesion** (to extracellular matrix components especially HA) and also homotypic **cell aggregation**.

CD44 has been called as H-CAM (Hyaluronate-cell adhesion molecule). Hyaluronan is seen in abundance in extracellular matrix and act as principle ligand for CD44, but it is not the only ligand. The other ligands include rest of extracellular matrix component like collagen, chondroitin sulphate, fibronectin and laminin.

Stroma of most of the tumors contain increased amount of hyaluronic acid. Hence increased expression of CD44 by the neoplastic cells potentiates the tumor cells to adhere to extracellular matrix through ligands like hyaluronan and allows active formation of cell colonies.

In addition, over-secretion of CD44 by tumor cells might cause the tumor cells to be perceived as lymphocytes by the immune system, which might trigger the occurrence of immune escape (13). CD44 thus secreted binds to HA, and during the transport of HA, it also intervenes in the transportation of tumor cells into the lymphatic flow (11).

CD44 also functions as a putative cancer stem cell surface marker and henceforth can be used as a novel therapeutic target. CD44 has the property to internalize hyaluronan and therefore can act as a target receptor for

hyaluronan-conjugated drugs, nanocarrier delivery systems, or for anti-CD44 antibodies linked to radioactive isotope or chemotherapeutic agents.

An increased levels of soluble CD44 was found in the serum of patients with gastric carcinoma which may be used as a prognostic indicator of tumor burden and metastasis in such cases(47).

The different methods for evaluating the presence and degree of CD44 expression includes immunocytochemistry, which helps to detect the localisation of CD44 molecule in tissues, others being fluorescence cell sorting and reverse transcriptase polymerase chain reactions which will help in quantitative analysis by measuring mRNA levels of CD44.

OZMEN F et al (13) studied tissue samples from 33 patients (8 females) with gastric cancer. Quantitative and qualitative analysis of the following markers like LYVE-1, VEGFR-3 and CD44 were analyzed by both RT-PCR and IHC. 64% of cases showed CD44 positivity. No correlation was made between CD44 expression and age, sex data's. CD44 expression was showing significant statistical correlation with histological type, grade and stage of the tumor. Increased expression of CD44 was associated with increased risk of metastasis and poor survival in this study.

YUTA WAKAMATSU Y (15) and his colleagues evaluated a total of 190 cases of gastric carcinoma. They analyzed the expression of cancer stem cell markers like ALDH1, CD44, and CD133 in both the primary tumors as well as in lymph node metastasis of gastric cancer by immunohistochemical method.

Out of 190 primary gastric carcinoma cases studied, 117(62%) were positive for CD44 and rest of the two markers showed less apparent expression compared to CD44.

Expression of all the three cancer stem cell markers studied showed significant correlation with advanced clinicopathologic factors.

Patients with both CD44 and CD133 positive gastric carcinoma had a poorer survival rate than the patients with both markers negative. CD44 expression and CD133 expression are considered as independent predictors of survival in patients with gastric carcinoma(15)

HAMID et al (36) studied 100 cases of gastric adenocarcinoma for its expression of CD44 by immunohistochemistry. 65% of tumors were CD44 positive, which were most commonly associated with intestinal type of tumors. A strong statistical significance was seen to exist between CD44

expression and the histological grade of tumor. CD44 positive tumors showed poorer survival .

STREIT M et al (37) in their study identified that, occurrence of the CD44 standard and the CD44-9v isoform on the gastric cancer cells was significantly associated with higher tumor-induced mortality and also shorter survival time. The intestinal-type of gastric carcinomas shows high CD44v6 expression.

YOON H KO et al(38) reviewed 159 cases of non small cell lung cancer. He investigated CD44s protein expression in 159 cases by using tissue array. High CD44s expression was detected more with squamous cell carcinoma (91.7%) than in those with adenocarcinoma histology.

XIN, YAN M.D (39) and his other expertise colleagues reviewed nearly 150 cases of gastric adenocarcinomas of which 100 were advanced gastric cancers, 36 were early gastric cancers and the rest were of intermediate category. CD44V6 expression was studied using immunohistochemistry. Increased expression was showing strong association with advanced cases and also lower survival rate.

SHA L et al analysed the rate and mean fluorescence intensity of CD44 cells by flow cytometry. In gastric carcinoma, increase in CD44 protein indicates its major role in the invasion and metastasis of gastric carcinoma.

MAYER B (40) and his colleagues attempted to study the prognostic value of CD44 in gastric adenocarcinoma. They evaluated both benign (59) and malignant (primary 61, metastatic 59) gastric tissue for its expression for monoclonal antibodies against CD44. CD44 negative in normal mucosa and shows positivity only in malignant tissue. 49% of cases showed CD44 positivity which was expressed more in cases of primary tumor who underwent curative resection.

CD44 positive cases showed increased tumor recurrence and increased mortality during follow-up. Good correlation was made out between the expression of total CD44 and tumor recurrence and mortality.

CHANG HAK YOO M et al(41) conducted a prospective study of total of 261 cases of gastric carcinomas. Tissue samples from all the cases were stained with the monoclonal antibodies against both CD44 and nm23 by IHC method. 43.2% of cases showed CD44 positivity. Significant difference in five year survival rate was made between CD44-positive and

negative cases (P = 0.0018). However, there was no such difference seen between patients with nm23-positive and negative cases.

CHING-SHYA YONG (42) and his colleagues conducted a retrospective study. They reviewed the hospital records of 500 patients with gastric cancer who underwent surgical resection. Out of 500 patients, 95 patients developed recurrence post operatively. Twenty patients from the recurrence group and 20 patients from the non-recurrence group were randomly selected and identified as study and control groups, respectively.

They analysed expression of both CD44 and CD24 protein in both the groups by immunohistochemistry. The study revealed that the expression of CD44 protein was significantly correlated with stage of the tumor than that of CD24.

KARL-HEINZ HEIDER et al (43) evaluated 42 cases of gastric adenocarcinomas for CD44 expression by IHC. Study revealed that adenocarcinomas of the intestinal type were strongly positive for CD44v6, whereas diffuse-type adenocarcinoma showed CD44v5. Analysis of RNA expression confirmed the data obtained with immunohistochemistry. These differences in expression was attributed to different origins of these tumor

types. It was concluded that CD44 can be used as a tool in gastric cancer prognosis.

YOO, C. H et al (44) and his team studied about a total of 261 cases of gastric carcinomas considering only stage II and stage IIIA. Immunohistochemical evaluation of tissue samples by staining with the monoclonal antibodies against CD44 and nm23. Of 261 cases studied 31.0% were CD44 positive and 70.1% showed nm23 positivity.

No significant association was made between CD44 expression and clinicopathological variables. But the 5-year survival rates between CD44-positive and negative cases was found to be significant ($P = 0.0018$).

SADHNA DHINGRA et al (45) and the team members evaluated the expression of cancer stem cell markers like CD44 and nestin in both neoplastic and also in non-neoplastic gastric tissue. Tissue microarray was structured by tissue samples obtained from 174 cases of gastric adenocarcinoma and 41 samples of adjacent non neoplastic gastric mucosa. Clinical data's were procured. Expression of CD44 and nestin were assessed by immunohistochemistry. The study revealed 51% (78/152) of CD44 positivity and 25% (43/174) of nestin positivity. CD44 positivity was

significantly greater in gastric adenocarcinoma ($P<0.001$) and more frequently associated with Lauren's intestinal histologic subtype ($P<0.05$).

YAMAGUCHI et al (46) found that the expression of CD44v6 protein was significantly higher in differentiated adenocarcinoma than in diffuse-type carcinoma.

SAITO et al (47) observed in his study that high CD44v6 expression is seen in association with intestinal-type gastric carcinoma.

IMMUNOHISTOCHEMISTRY

IMMUNOHISTOCHEMISTRY

Immunohistochemistry as the name suggests, involves the principles of two divisions which are nothing but immunology and histology. It is defined as the technique for identifying antigens by means of antigen-antibody interaction, the site of interaction being detected by either primary labeling of the antibody or by secondary labeling method. (35)

This technique determines whether the tissue under study expresses a particular antigen and in addition to that it also determines the antigenic status of particular cells within that tissue and its cellular location by which it identifies the biology and lineage specificity(36).

The key to immunohistochemistry is the specificity of antibodies for particular antigens. The subjectivity which is common in any surgical pathology diagnostic procedures may lead to common problem of lack of reproducibility among the pathologists.

Much of the variations in immunohistochemical test results are mainly due to irregularities in specimen handling, particularly fixation. It is

generally noted that many of the antigens are severely affected by long duration of fixation. It is commonly advised that the duration of fixation (standard is formalin fixative) must be restricted to a maximum of 24 hours.

It has been emphasised that the immunohistochemical studies done in absence of proper controls are considered of no value and could even be dangerous. Any departure from the protocol which is recommended by the manufacturer, necessitated the revalidation of whole technique by the performing laboratory.

Detection system (35)

Antibodies are labeled to allow visualization of the site of reaction-which include fluorescent substances(fluorescent microscopy), enzyme labels (eg. Horse radish peroxidase) forming colored reaction with suitable substrate(light microscopy) , heavy metals (electron microscopy) and even radiolabels but requires autoradiographic facilities.

The different techniques of immunohistochemistry is as follows:

1. **TRADITIONAL DIRECT TECHNIQUE** – Primary antibody is directly conjugated with the label which then binds with the antigen in the tissue.

2. **NEW DIRECT TECHNIQUE(ENHANCED POLYMER ONE STEP STAINING METHOD)**- Here the large number of primary antibodies and enzyme labels are tagged to a dextran polymer which acts as backbone which increases the signal and better sensitivity of the test.

3. **TWO -STEP INDIRECT TECHNIQUE**- Here the secondary antibody will be labeled against the immunoglobulin of the animal species in which primary antibody been prepared. This is more sensitive than the direct method in detecting antibodies.

4. **UNLABELLED ANTIBODY-ENZYME COMPLEX TECHNIQUE** (Peroxidase-Antiperoxidase complex)

5. **IMMUNOGOLD SILVER STAINING TECHNIQUE**- This is used in ultrastructural immunolocalisation. Gold particles are enhanced by the addition of several layers of metallic silver.

6. **STREPT(AVIDIN)-BIOTIN TECHNIQUE**- Here avidin is conjugated with secondary antibody and similarly biotin with the primary antibody has been used based on the fact of existence of high affinity binding between both avidin and biotin.

7. HAPTEN LABELLING TECHNIQUE.

Different methods of antigen retrieval techniques has been demonstrated which includes,

1. Proteolytic enzyme digestion method
2. Microwave antigen retrieval method
3. Pressure cooker antigen retrieval method
4. Water bath method.

These techniques allow unmasking of antigens which was happened during fixation.

MATERIALS AND METHODS

MATERIALS AND METHODS

PATIENT SELECTION:

The protocol for this study was reviewed and approved by the Institutional Ethical committee, Govt. Stanley Medical College, Chennai. Data was obtained retrospectively from registers which include the patients data who underwent gastrectomy (subtotal/total) for gastric cancer from August 2011 to August 2012. A retrospective review of patient's medical records was obtained and data were collected for age, sex, presenting symptoms, socioeconomic status (assessed by occupation, literature and per capita income), risk factors like smoking, alcohol and tobacco chewing (50) and also the final pathologic diagnosis with the help of prestructured proforma.

Tumors were classified histologically according to the WHO criteria and was staged according to TNM staging; Lauren's classification system was applied for histological typing of tumor (intestinal type gastric cancer corresponds to well- or moderately differentiated tumors; diffuse type corresponds to poorly differentiated tumors).

Clinical history and investigation findings were tabulated.

STUDY DESIGN- Longitudinal retrospective study.

EXCLUSION CRITERIA:

1. Non adenocarcinoma cases.
2. Patients who underwent preoperative chemotherapy.

INCLUSION CRITERIA:

Fifty patients with histologically verified diagnosis of gastric adenocarcinoma were randomly (cases with complete clinical data) selected as subjects in my study and was retrospectively analyzed for CD44 expression in patient's post-operative pathologic specimens.

TISSUE PREPARATION

Formalin fixed paraffin embedded blocks of the cases of gastric adenocarcinoma included in this study were taken. H&E sections of these cases were reviewed and analysed both for grading and staging. Appropriate blocks were chosen for further evaluation of CD44 IHC staining.

IHC was done by HRP polymer technique.

1. Chrom alum coated slides were prepared for IHC examination and 4 micron thick sections were taken in chrom alum coated slides and incubated for atleast 2 hours.
2. After paraffin removal and rehydration, antigen retrieval was performed by placing sections into a beaker containing adequate amounts of citrate buffer (pH 6.0), then heating in microwave oven for 15 minutes and cooling to room temperature.
3. Peroxidase block was added for 30 minutes, for inhibiting endogenous peroxidase in the tissue.
4. Sections were washed in TRIS buffer for 5 minutes.
5. Power block was applied for 30 minutes which blocks non specific antigen antibody reaction.
6. Excess power block was blotted.
7. Primary antibody (mouse anti-CD44 monoclonal antibody, clone DF1485 which identifies all isoforms of CD44) was applied for 60 minutes.
8. Washed in TRIS buffer for 5 minutes twice.

9. Applied super enhancer for 30 minutes which enhances the final reaction product by increasing the sensitivity of antigen antibody reaction.
10. Secondary antibody was then added for 15 min, followed by TRIS buffer washing .
11. DAB substrate solution was subsequently added and washed with running water followed by hematoxylin counterstaining.
12. Finally, tissue sections were washed with distilled water, dehydrated and mounted for microscopic evaluation.
13. IHC analysis was done for all cases and scored for each case.

Tonsillar tissue was used as positive control and in CD44 negative cases, the tumor infiltrating lymphocytes present in neoplasm was used as internal positive control and normal gastric tissue was used as negative control.

Scoring technique was as follows- For the interpretation of CD44 expression in the tissue, both the staining intensity and percentage of staining area were considered.

1. Intensity of staining was scored as follows:

(0)= No staining

(1+)= Weak staining

(2+)= Moderate staining

(3+)= Strong staining

2. The **percentage of staining area** was scored accordingly :

0= 0%;

1=when 1-10% of the area gets stained,

2=when more than 10%, but upto 50% of the tumor shows staining,

3=more than 50% but upto 100% of the tumor are being stained.

C**omposite score** was obtained by multipying both the intensity of staining as well as the percentage of staining. The composite score ranges from 1 to 9. Composite scores of 1-3 was labelled as 'low CD44 expression', whereas scores of 4-9 were labelled as "high CD44 expression".

Tumors with both weak and moderate / strong staining is regarded as positive expression, while those with negative staining only is considered as negative expression.

Statistical analysis were performed to correlate between the CD44 expression status and clinical variables such as age, sex, socioeconomic status, risk factors like smoking, alcohol and tobacco chewing, location of tumor and also histologic parameters like histological type, grade and stage. Results were tabulated and statistical significance analysed for necessary variables such as CD44 expression against histological type, grade and stage. P value of less than 0.05 was considered statistically significant.

OBSERVATION AND RESULTS

Table 1. Age And Sex Distribution In Gastric Adenocarcinoma

AGE	MALE	FEMALE	TOTAL NUMBER OF CASES
	Number of cases	Number of cases	
0-20	0	0	0
21-40	2	2	4
41-60	12	6	18
>60	20	8	28
TOTAL	34	16	50

Out of 50 cases of gastric adenocarcinoma studied 34(68%) were males and 16(32%) were females with Male:Female sex ratio accounting to 2.1:1. Among the 50 cases studied, majority (56%) were in the age group of above 60 years and gastric adenocarcinoma was found to be more prevalent among people above 60 years.

Table 2. Presenting symptoms of 50 cases (gastric adenocarcinoma)

SYMPTOMS	NUMBER OF CASES	PERCENTAGE OF CASES(%)
DYSPEPSIA	47	94
EPIGASTRIC PAIN	40	80
VOMITING	12	24
MALENA	4	8

Dyspepsia, followed by epigastric pain was found to be the common presenting symptoms seen in majority of cases. Vomiting was seen in most of the advanced stages of cancer. Malena was found in cases with ulcerative tumor morphology.

Table 3. Socioeconomic Status Of 50 Patients Presented With Gastric Adenocarcinoma

SOCIOECONOMIC STATUS	NUMBER OF CASES
LOWER	7
UPPER LOWER	40
LOWER MIDDLE	2
UPPER MIDDLE	1
UPPER	0
TOTAL	50

Out of 50 cases, majority of (80%) patients belonged to lower socio economic status.

Table 4. Assessment Of Risk Factors Like Smoking, Alcohol Intake, Tobacco Chewing In 50 Cases Studied

RISK FACTORS	MALE	FEMALE
SMOKING	8	0
ALCOHOL	12	0
TOBACCO	5	6

Of the 50 cases evaluated smoking and alcohol appears as major factors in males. Tobacco chewing was significantly associated with female population. Tobacco chewing was included in the list to demonstrate the association of it with gastric carcinoma, as smoking and alcohol among females was not so common in South Indian countries.

Table 5. Anatomical Distribution Of Gastric Adenocarcinoma With Respect to Sex distribution In The 50 Cases

SITE	FEMALE	MALE	TOTAL
CARDIA	2	9	11
FUNDUS AND BODY	5	13	18
ANTRUM	9	12	21

Out of 50 cases, predominant number(21) of cases showed tumor at the site of antrum, followed by body/fundus(18) and least number of cases seen in cardia(11) region. No significant association was made between anatomical location of tumor with respect to sex of the patients.

Table 6-Tumor morphology in 50 cases of gastric adenocarcinoma

TUMOR MORPHOLOGY	NO. OF CASES
PROLIFERATIVE	41(82%)
INFILTRATIVE	9(18%)

Most of the tumors in the present study showed ulceroproliferative growth than compared to infiltrative tumor morphology.

Table 7. Histological Type Of Gastric Adenocarcinoma (Laurens Classification)

HISTOLOGICAL TYPE	NUMBER OF CASES	% OF CASES
Intestinal type	43	86
Diffuse type	7	14
Total	50	100

Among the 50 cases studied, 43 of them showed intestinal type and 7 of them showed diffuse type- indicating the predominant pattern of adenocarcinoma being intestinal type.

Table 8. Histopathological Grading Of 50 Cases Of Gastric Adenocarcinoma In This Study

GRADING	NO. OF PATIENTS	% OF CASES
WELL DIFFERNTIATED	7	14
MODERATELY DIFFERENTIATED	31	62
POORLY DIFFERENTIATED	12	24
TOTAL	50	100

Histological grading of gastric adenocarcinoma in these 50 cases revealed 31 cases with moderately differentiated adenocarcinoma, followed by poorly differentiated tumors accounting to number of 12 cases and only 7 showed well differentiated carcinoma.

Table 9. TNM Staging Of Gastric Adenocarcinoma Cases In This Study

HISTOLOGICAL STAGE	NO. OF CASES	% OF CASES
Stage I	10	20
Stage II	11	22
Stage III	25	50
Stage IV	4	8

Histological staging of gastric adenocarcinoma in these 50 cases revealed 25 cases in stage III, 11 cases in stage II and 10 cases in stage I cancer. 4 cases were found to be stage IV. This implies that most of the cases when they present with signs and symptoms has already been in advanced stage.

Table 10. Histological grade versus Stage in 50 cases

	DIFFUSE TYPE	INTESTINAL TYPE
WELL DIFFERENTIATED	0	7
MODERATELY DIFFERENTIATED	0	31
POORLY DIFFERENTIATED	7	5
TOTAL	7	43

Almost all the diffuse type(7) of gastric carcinoma was poorly differentiated, whereas intestinal type showed all three differentiation viz. well / moderate / poor in varying proportions.

Table 11. CD44 Expression In 50 Cases Of Gastric Adenocarcinoma

TOTAL NO. OF CASES	NO. OF CASES	% OF CASES
CD44 POSITIVE	43	86
CD44 NEGATIVE	7	14

Out of 50 cases, 43 cases showed CD44 positive expression and 7 showed negativity to CD44.

Table 12. CD44 Expression In 50 Cases Of Gastric Adenocarcinoma

CD44 EXPRESSION	NUMBER OF CASES	PERCENTAGE OF CASES
NEGATIVE	7	14
LOW EXPRESSION	23	46
HIGH EXPRESSION	20	40

Out of 43 CD44 positive cases, 23 cases showed low expression and rest of 20 cases showed high expression.

Table 13. Pattern Of CD44 Expression In Positive Cases

CD44 POSITIVE CASES					
LOW CD44 EXPRESSION			**HIGH CD44 EXPRESSION**		
CYTOPLASMIC	MEMBRANOUS	BOTH	CYTOPLASMIC	MEMBRANOUS	BOTH
9	12	2	0	15	5

Majority of the CD44 positive cases showed predominantly membranous pattern of staining, some showing cytoplasmic positivity and few shows both membranous and cytoplasmic staining. High number of membranous positive cases were seen among cases showing high CD44 expression.

Table 14. CD44 expression in relation to age and sex

AGE GROUP	CD44 EXPRESSION			
	Male		Female	
	Positive	Negative	Positive	Negative
0-20	0	0	0	0
21-40	2	0	2	0
41-60	10	2	5	1
>60	17	3	7	1

No significant correlation was seen between CD44 expression with respect to age and sex distribution among gastric carcinoma.

Table 15. CD44 expression vs anatomical location of gastric carcinoma

ANATOMICAL SITE	CD44	
	POSITIVE	NEGATIVE
CARDIA	9	2
FUNDUS AND BODY	15	3
ANTRUM	19	2

Table 16. CD44 Expression Vs Histological Type

HISTOLOGICAL TYPE	CD44 EXPRESSION		
	NEGATIVE	LOW	HIGH
INTESTINAL	6	17	20
DIFFUSE	1	6	0

CD44 expression is found to correlate significantly with intestinal type of gastric adenocarcinoma. The P value is 0.049 which is statistically significant.

Table 17. CD44 Expression Vs Histological Grade

CD44 EXPRESSION	HISTOLOGICAL GRADE		
	WELL DIFFERENTIATED	MODERATELY DIFFERENTIATED	POORLY DIFFERENTIATED
HIGH EXPRESSION	7	13	0
LOW EXPRESSION	0	15	8
NEGATIVE	0	3	4

CD44 expression was high in almost all cases of well differentiated adenocarcinoma, instead most of poorly differentiated tumors show low or negative expression only. The P value for this was 0.000 which was highly significant.

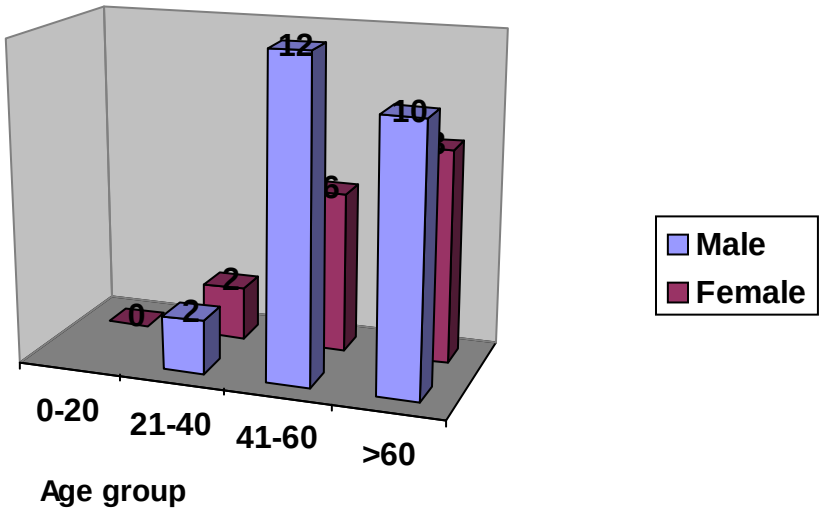
Table 18. CD44 Expression Vs Histological Stage

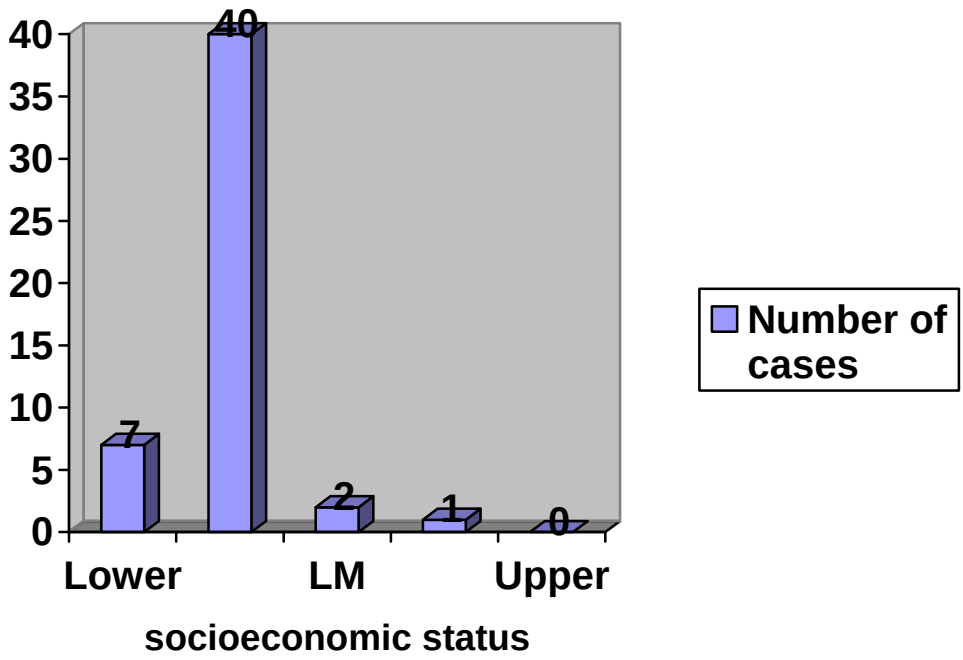
HISTOLOGICAL STAGE(TNM)	CD44 EXPRESSION		
	NEGATIVE	LOW	HIGH
	NO. OF CASES	NO. OF CASES	NO. OF CASES
STAGE 1	0	0	10
STAGE 2	0	5	6
STAGE 3	4	17	4
STAGE 4	3	1	0

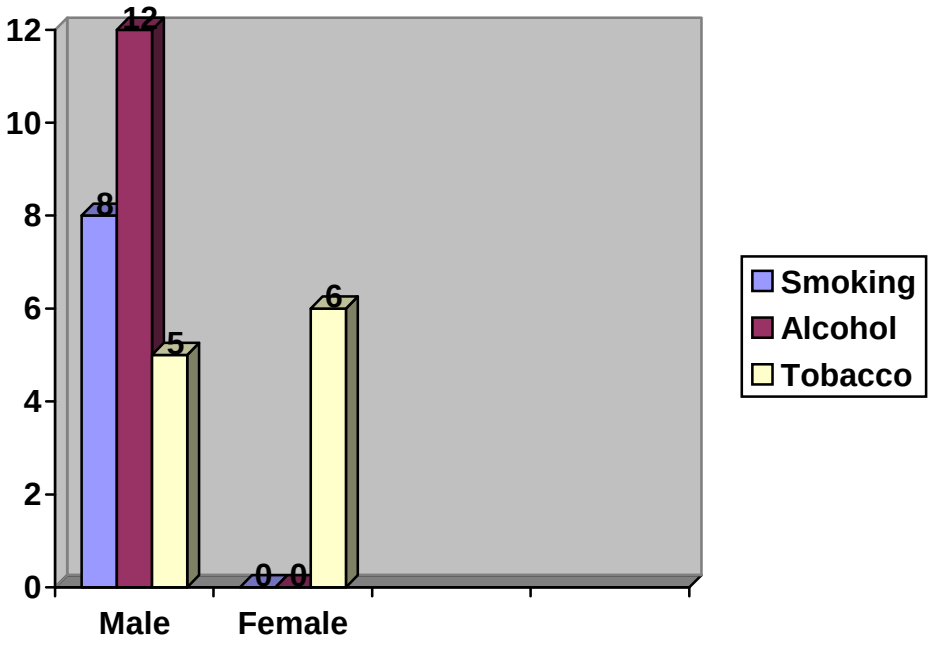
In the 50 cases analyzed, almost all the stage 1 tumors showed high expression, and most (3/4) of stage 4 tumors showed negative expression. The P value for this was 0.000 which was statistically significant.

Table 19. CD44 expression in relation to both histological grade and stage

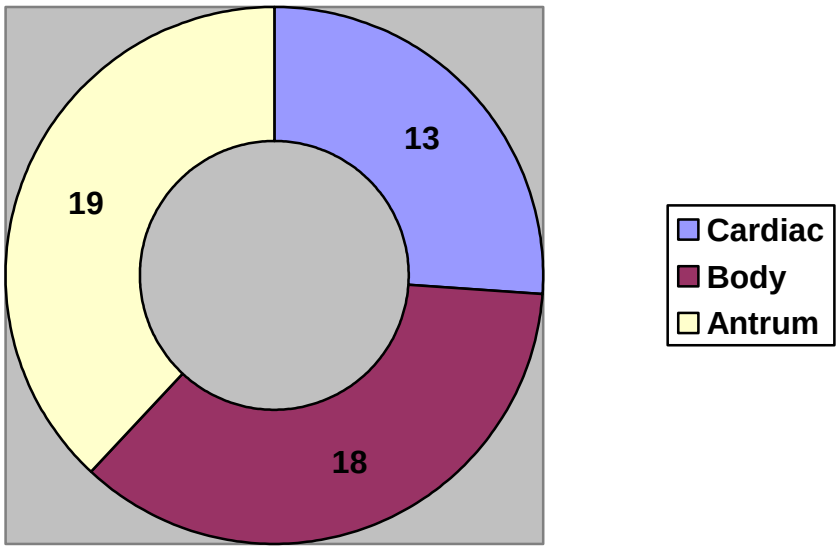
CD44 EXPRESSION	STAGE				GRADE		
	I	II	III	IV	WELL DIFF.	MOD. DIFF.	POORLY DIFF.
NEGATIVE	0	0	4	3	0	3	4
LOW	0	5	17	1	0	15	8
HIGH	10	6	4	0	7	13	0

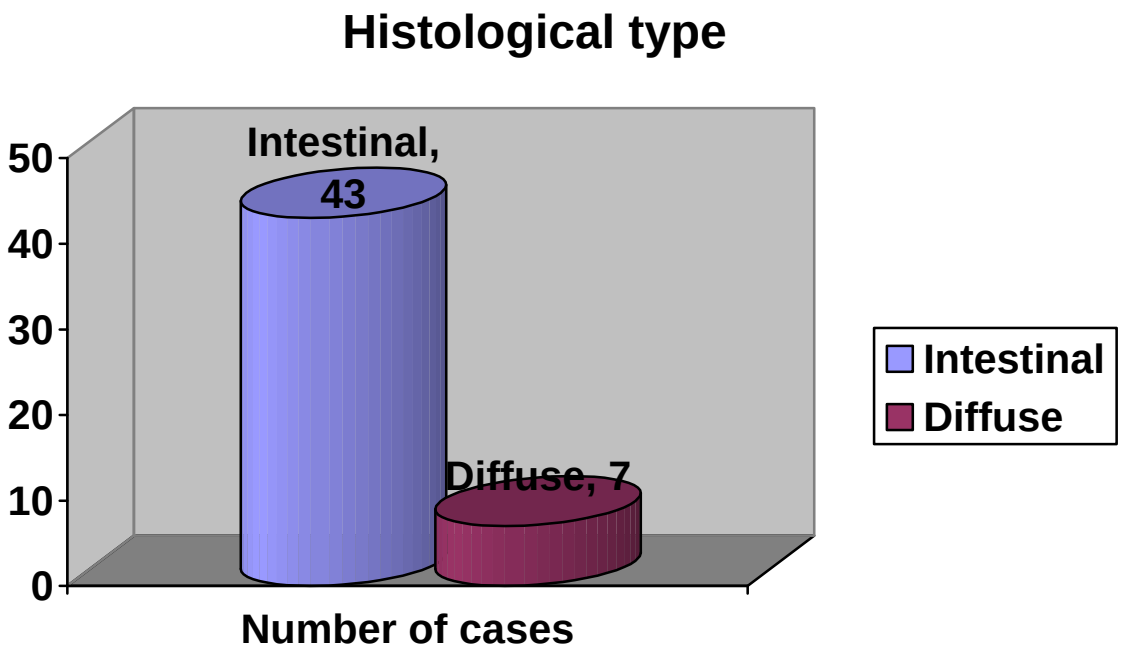

12
10
6
2
2
0
0-20
21-40
41-60
>60
Age group
Male
Female

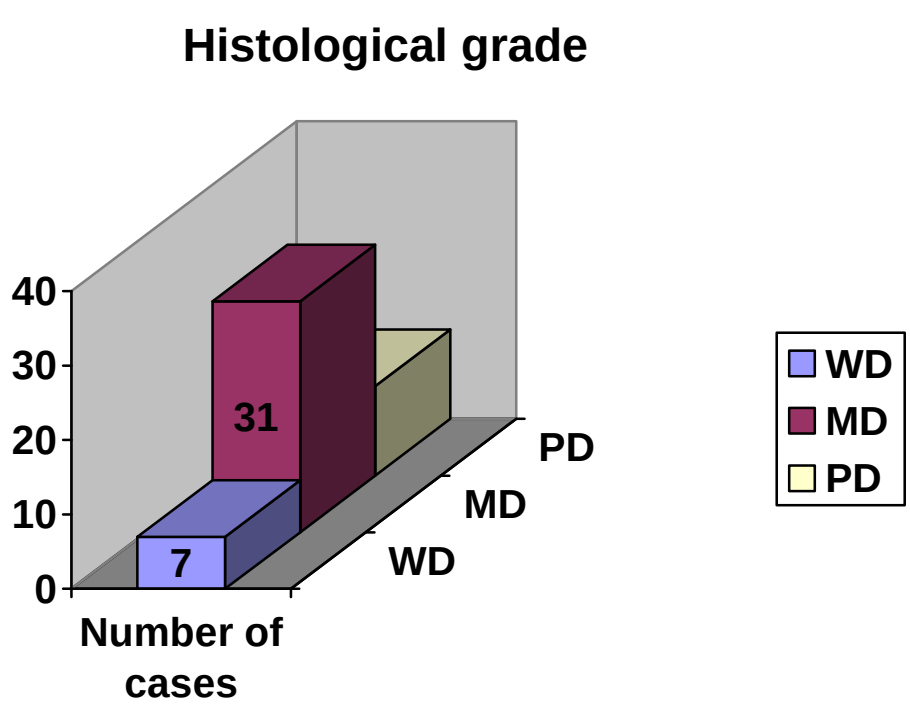

40
35
30
25
20
15
10
5
0
40
7
2
1
0
Lower
LM
Upper
socioeconomic status
Number of cases

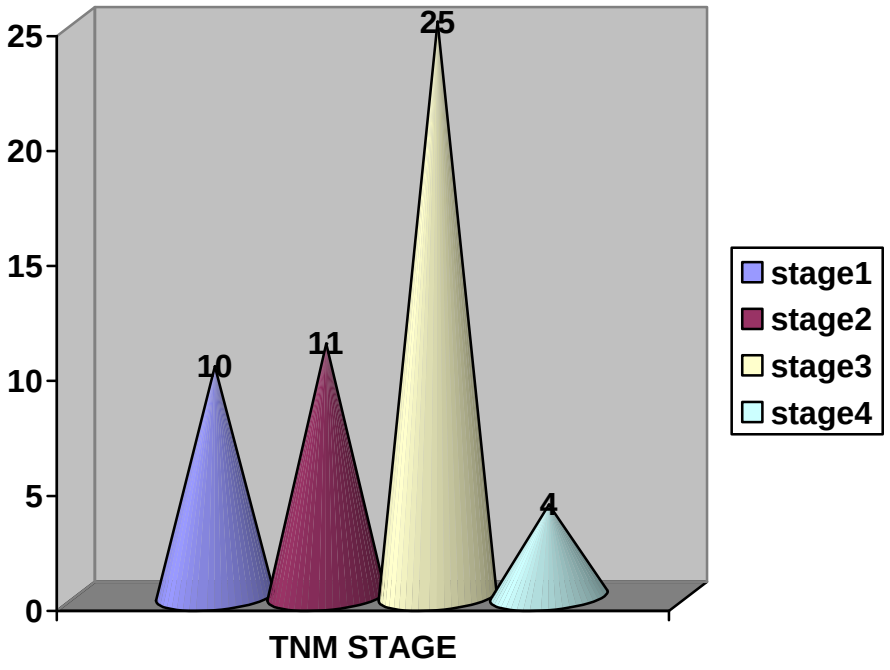


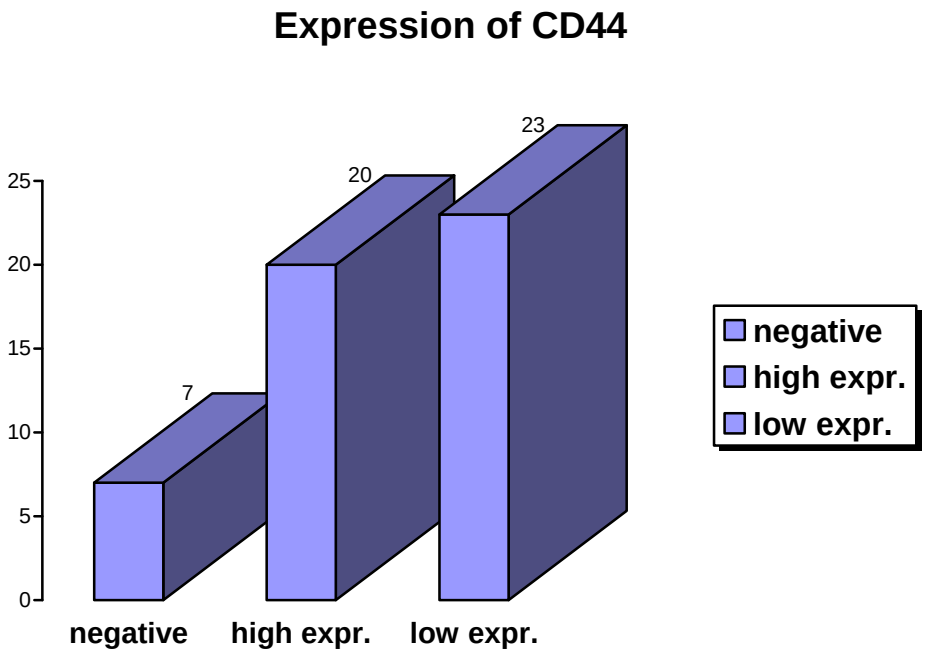
Location of tumor



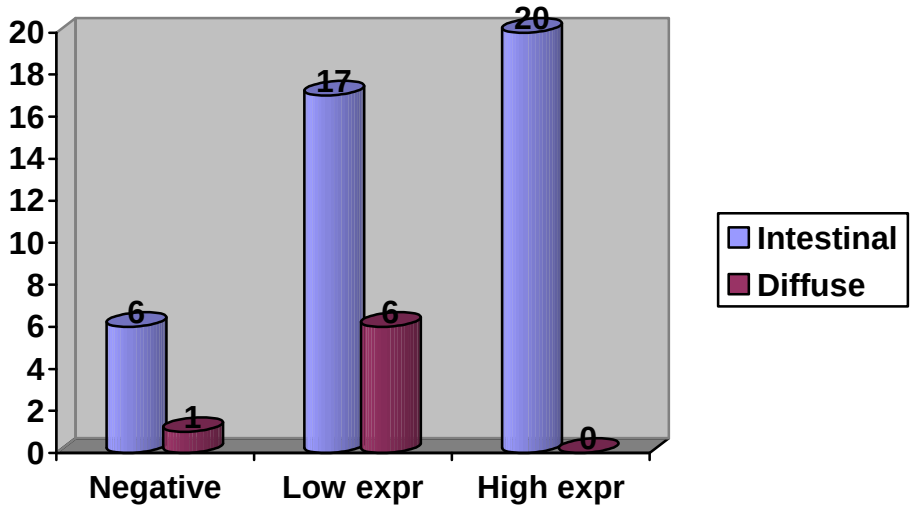

Histological type
50
40
30
20
10
0
Intestinal, 43
Diffuse, 7
Number of cases
Intestinal
Diffuse


Histological grade
40
30
20
10
0
31
7
PD
MD
WD
Number of cases
WD
MD
PD

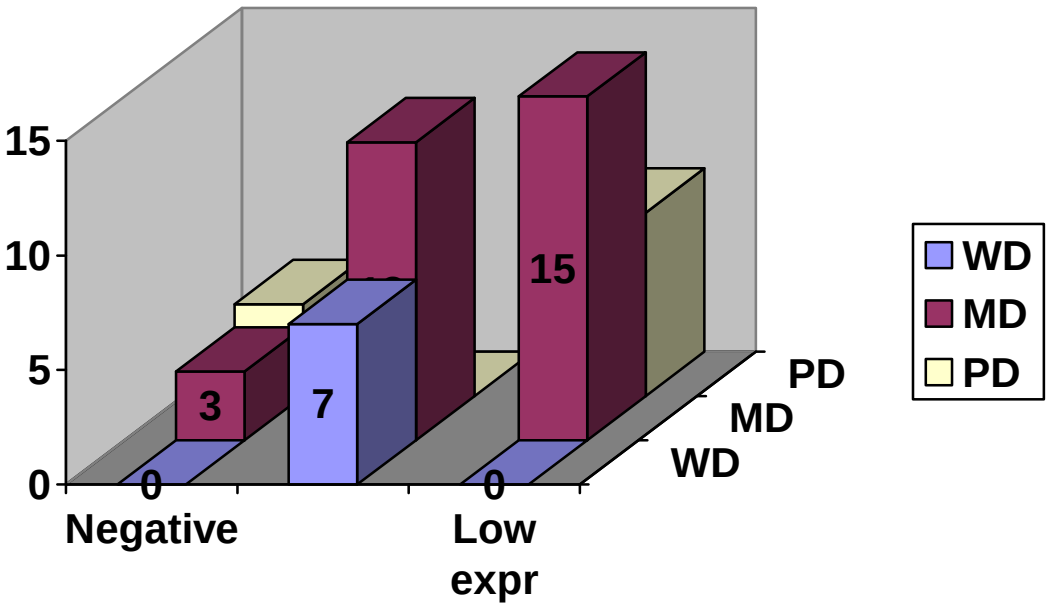

25
20
15
10
5
0
10
11
25
4
stage1
stage2
stage3
stage4
TNM STAGE

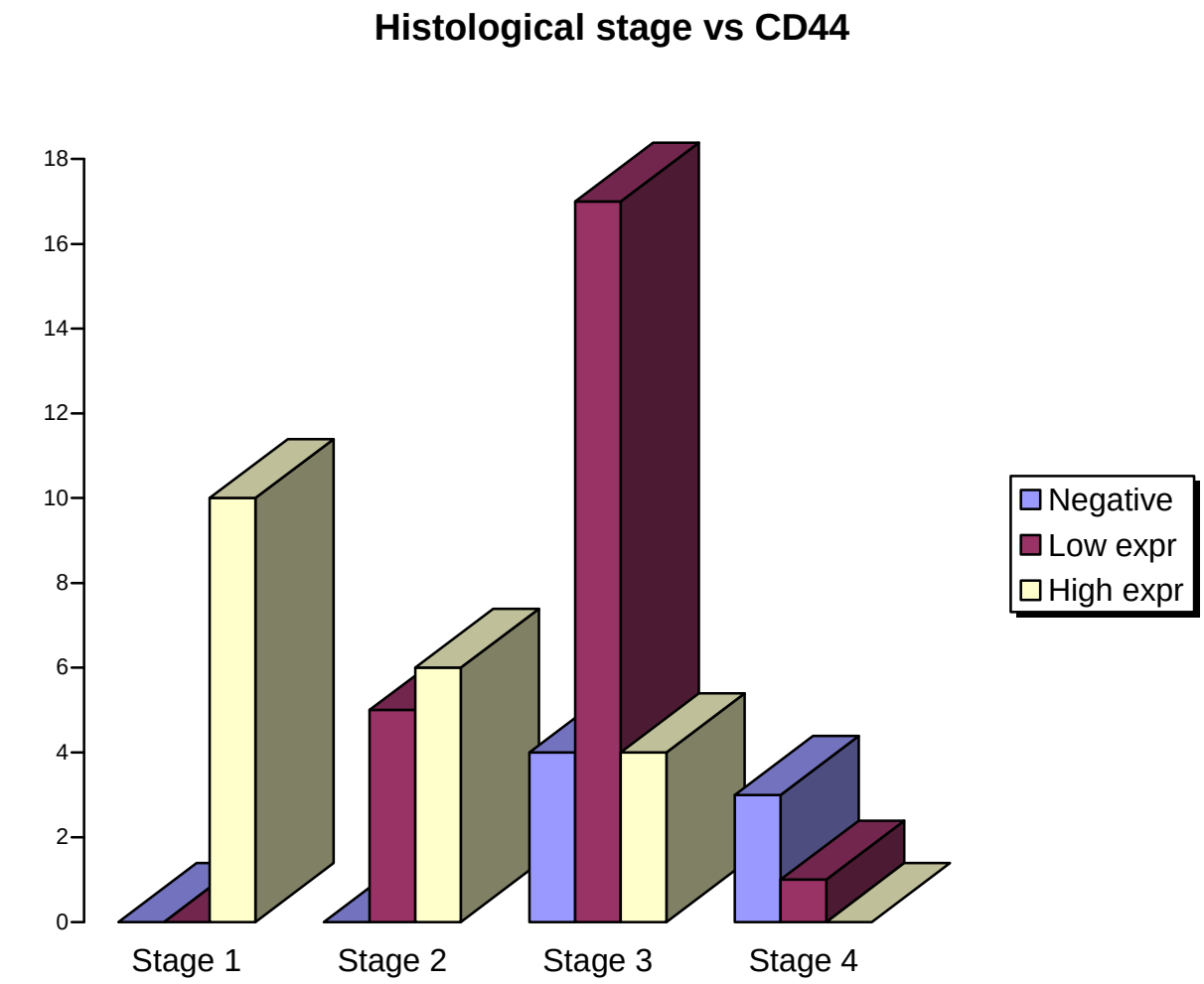

Expression of CD44
25
20
15
10
5
0
7
20
23
negative
high expr.
low expr.

Histological type vs CD44



Histological grade vs CD44

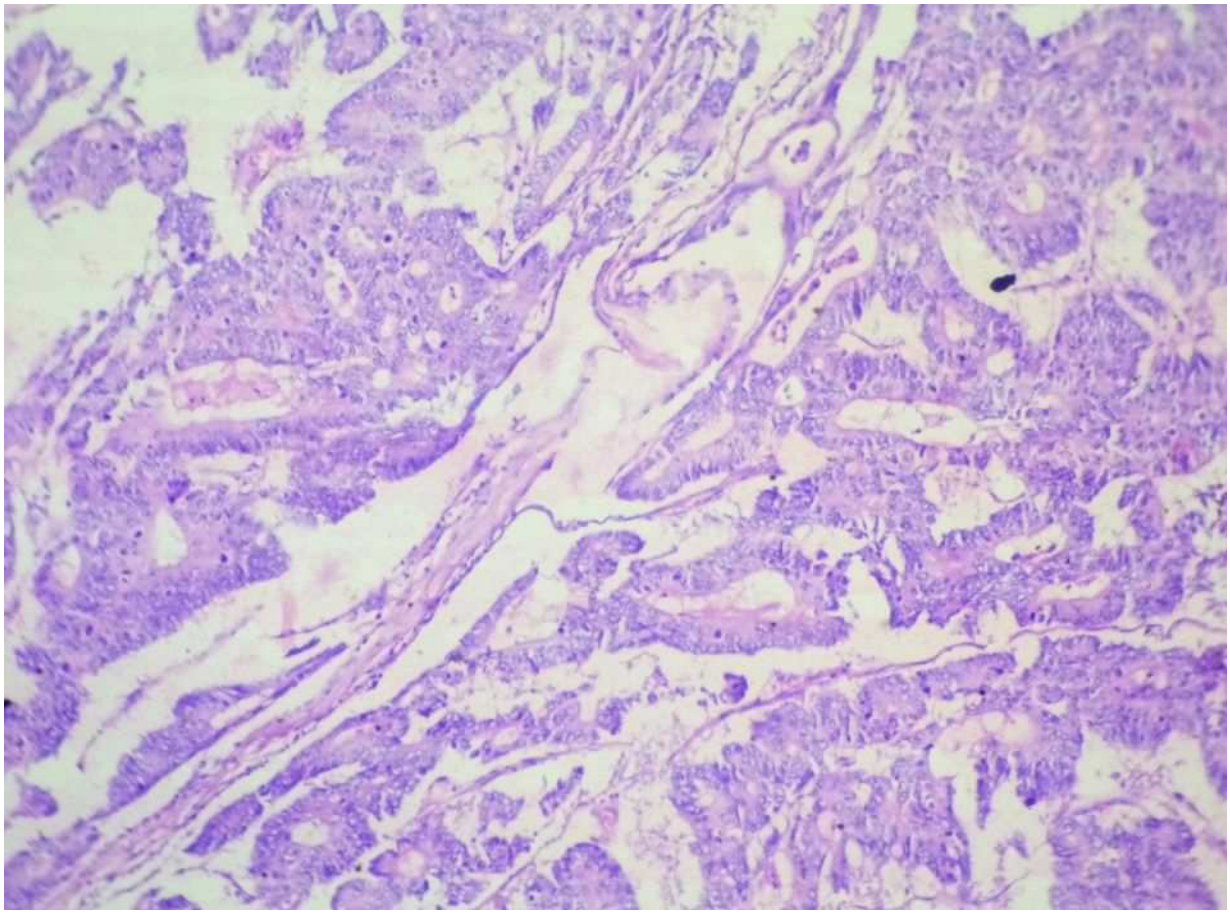



Histological stage vs CD44
18
16
14
12
10
8
6
4
2
0
Stage 1
Stage 2
Stage 3
Stage 4
Negative
Low expr
High expr

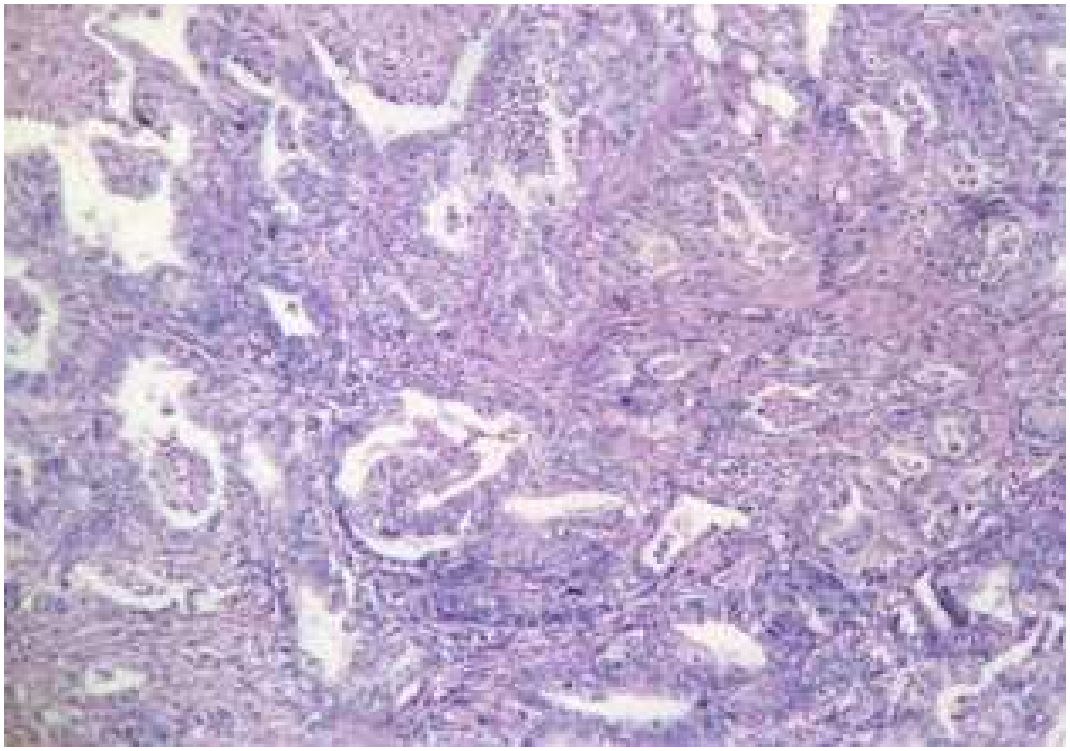
Total gastrectomy-Diffuse tumor involving the entire thickness.

Total gastrectomy specimen- proliferative growth in corpus

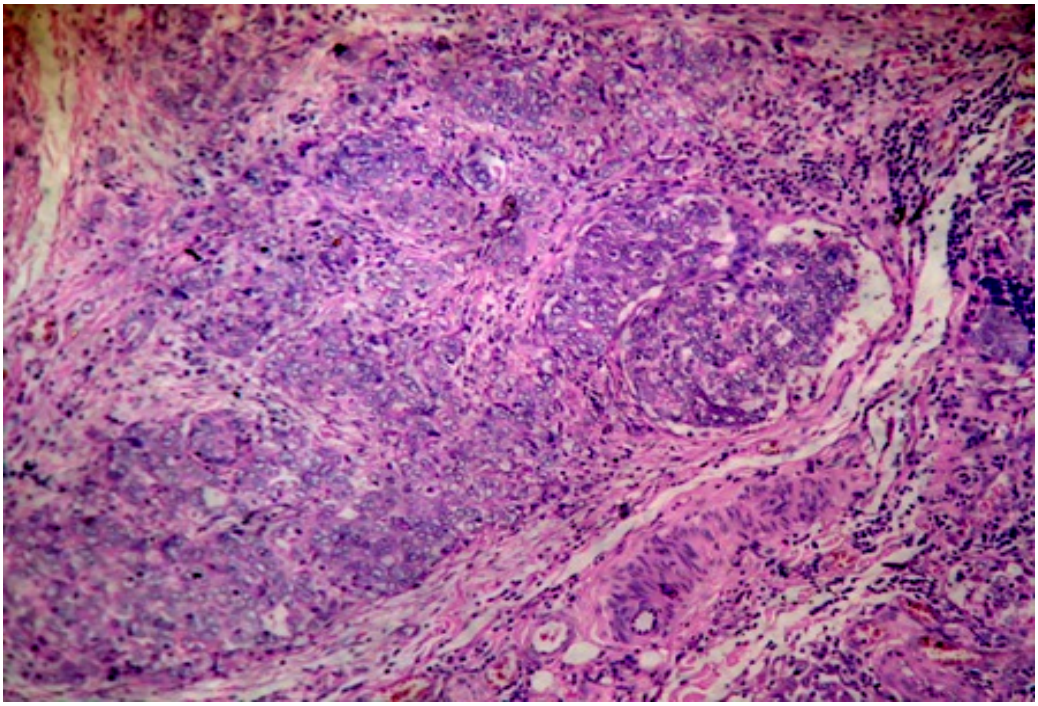
Total gastrectomy specimen-infiltrating tumor involving antrum.



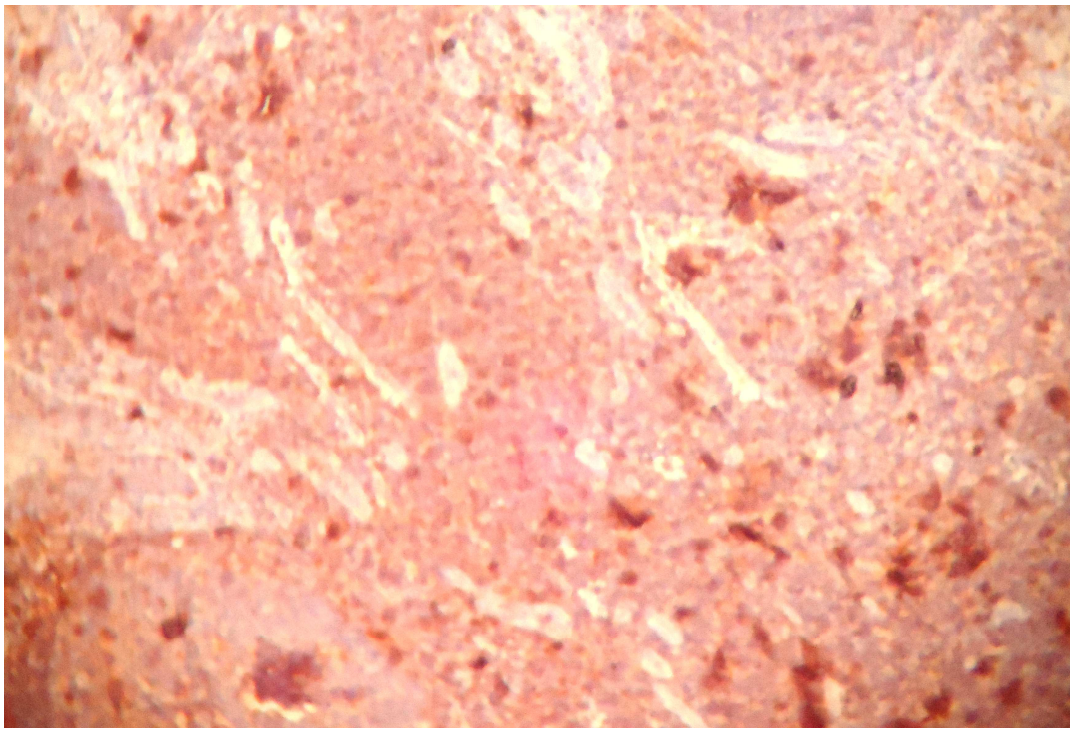
H&E-10X- Well differentiated adenocarcinoma-glands lined by neoplastic cells. Also seen are tumor infiltrating lymphocytes



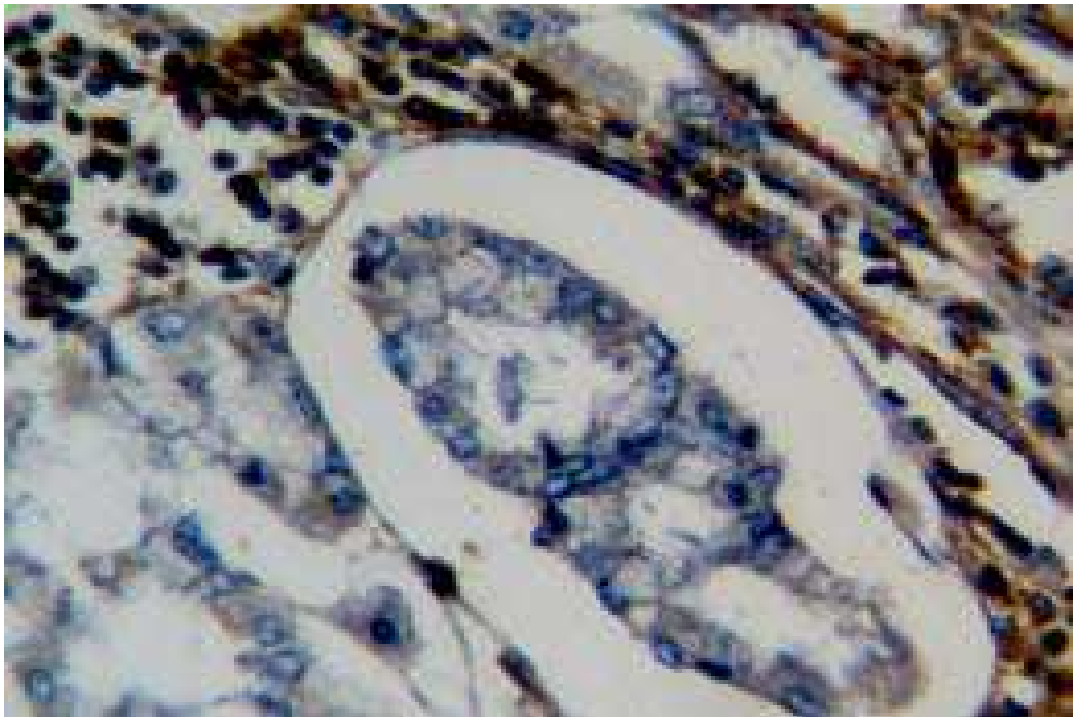
H&E-10X-Moderately differentiated adenocarcinoma-showing solid areas along with areas of gland formation.



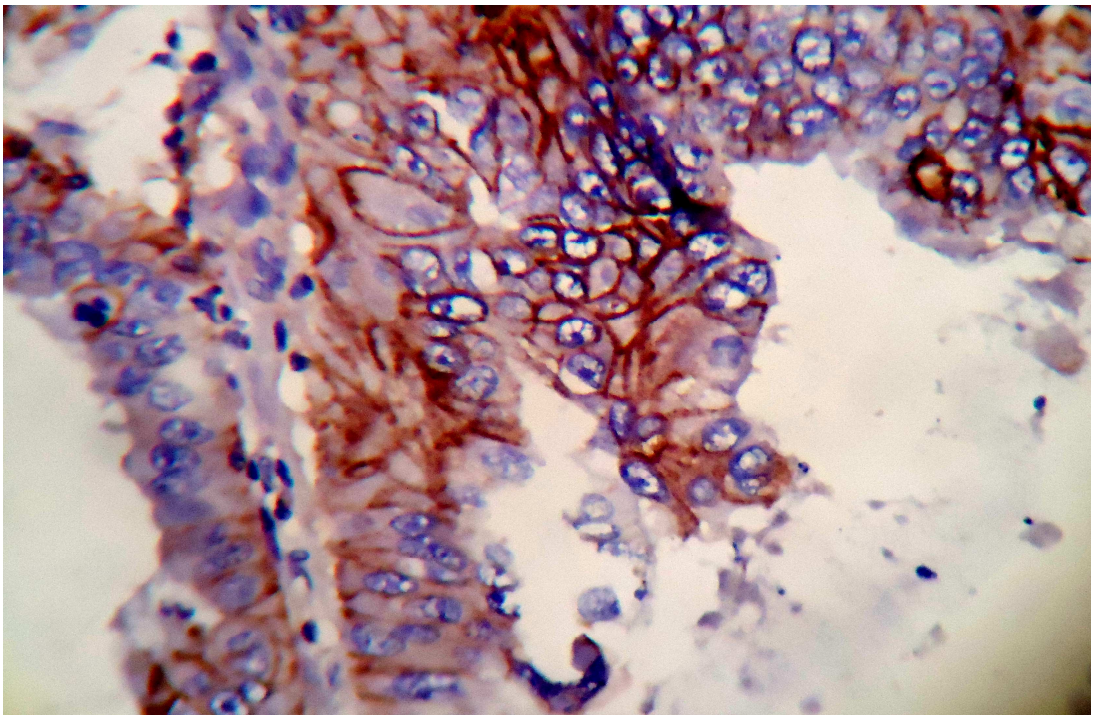
H&E-10X-Poorly differentiated adenocarcinoma- in solid sheets.



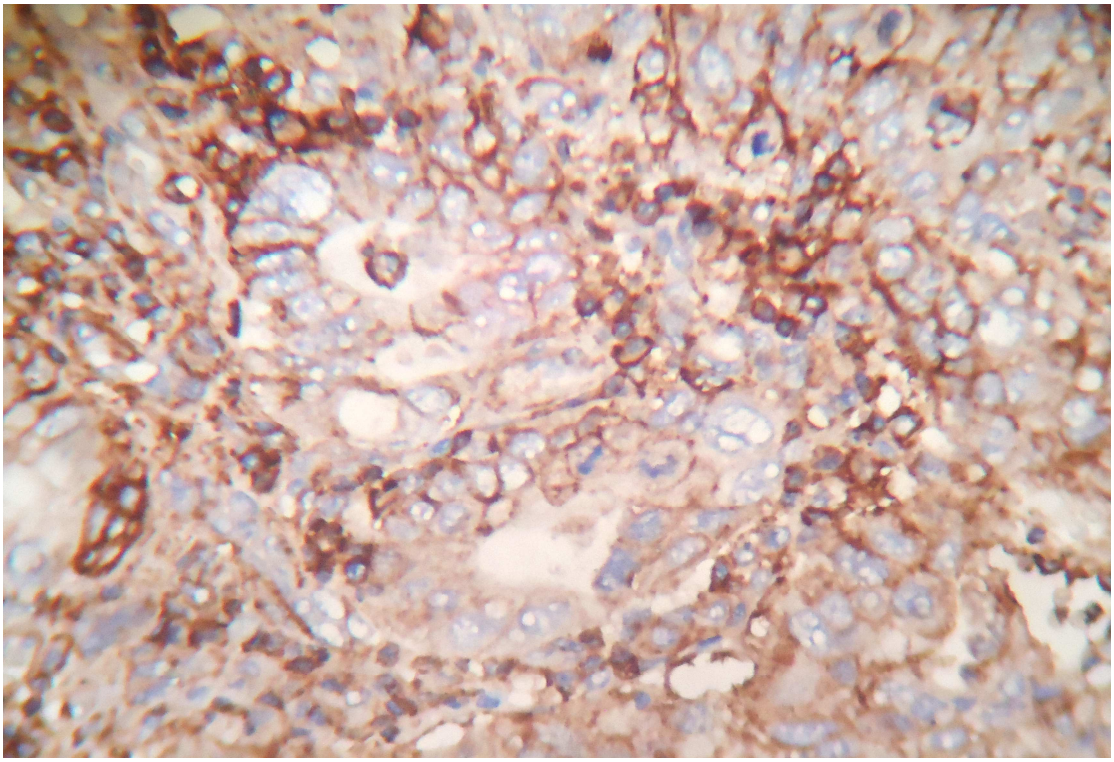
40X -Lymphocytes in the tonsil(control) with strong CD44 positivity.



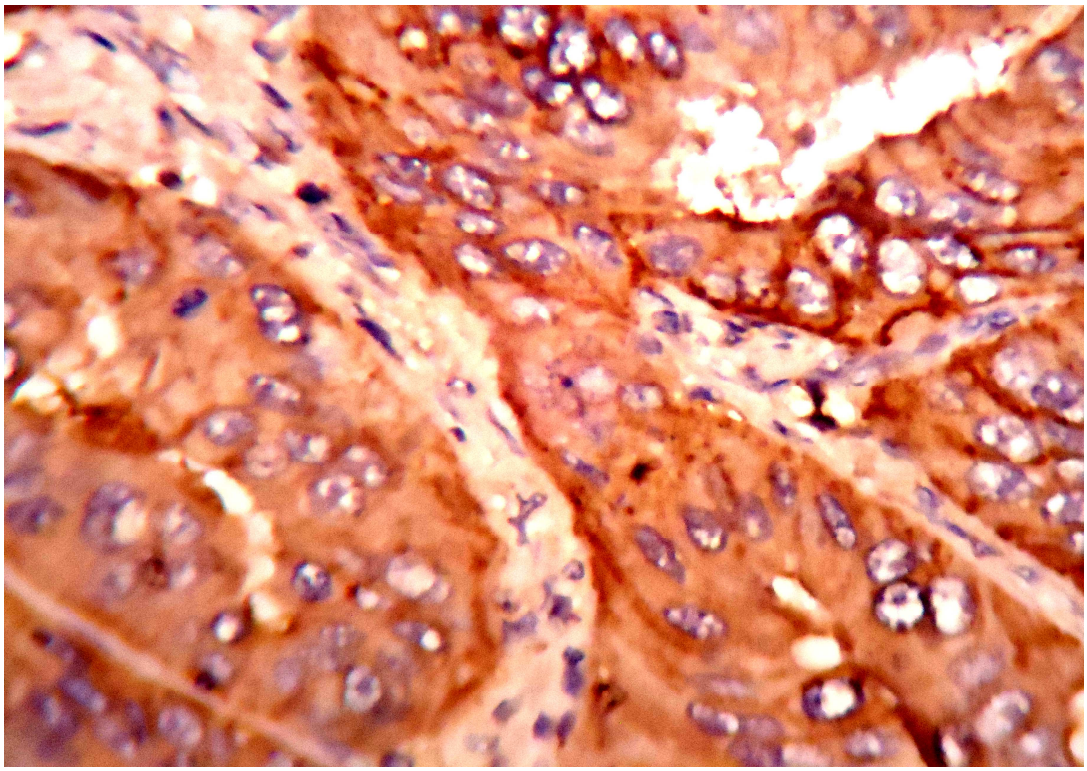
40X- CD44 negative in tumor glands but positive in in tumor infiltrating lymphocytes (internal positive control)



40X- High membranous expression of CD44 in tumor gland.



40X- Low membranous CD44 positivity in tumor glands.



40X- Both membranous and cytoplasmic CD44 positivity in tumor glands.

DISCUSSION

DISCUSSION

Gastric carcinoma is one of the leading cause of death worldwide. It is commonly seen after 40 years of age. Males are affected more than females. Risk factors that are very commonly seen in association with gastric carcinoma include lower socioeconomic status, smoking and alcohol consumption. Gastric antrum is the commonest site of tumor.

In accordance with this fact, the present study of 50 cases of gastric adenocarcinoma shows gastric carcinoma among the age range of 23 to 76 years with mean age of 49.5 years. Increased prevalence of gastric carcinoma was seen among the age group of more than 60 years(56%). Male/Female ratio of 2.1:1 was noted.

Significant association with lower socioeconomic status (80% belongs to lower socioeconomic status) as well as smoking and alcohol was also seen. This is due to the prevalence of H.Pylori infection in people of lower socioeconomic status. Most common presenting symptoms were dyspepsia (94%) and epigastric pain (80%).

Vomiting was seen in patients with advanced gastric carcinoma of proliferative (intestinal) type producing obstructive symptoms.

In most cases the tumors were located predominantly in antrum (42%).

Similar studies conducted in India showed the following results and were compared with the present study.

STUDIES	AGE GROUP	SEX	SITE OF TUMOR	SES
Chittukadu et al	55-64 years	M>F	Distal stomach	LOWER
Rajesh P et al	48 years	M>F	Distal stomach	LOWER
Bryan J et al	>40 years	M>F	Distal stomach	LOWER
Jijo velliyappillil cherian et al	>40 years	M>F with M:F=2.74:1	Antrum	LOWER
Present study	23-76 years (>60)	M(68%)>F with M:F=2.1:1	Antrum(42%)	LOWER

The histopathological parameters like histological type, grade and stage were also evaluated in the present study. It showed that intestinal type(86%) is more common than diffuse type which could be explained by its high prevalence in high risk regions. This study also showed increased occurence of moderately differentiated adenocarcinoma (62%) and most of them were reported to be Stage 3(50%).

Despite the lack of standardization and documented interobserver variation in the assessment, the histological grade has been shown repeatedly by multivariate analysis to be a stage-independent prognostic factor and it does not have any correlation with age or sex. In this study also there was no significant correlation of age and sex with histological grade of the tumor.

In view of better management / prediction of prognosis of the patients with gastric adenocarcinoma, development of new molecular biomarkers is necessary to assess the outcome of those patients so that the surgeons would manage them with intense therapeutic regimens (either medical/ surgical).

All the 50 cases which were included in present study were evaluated for CD44 expression which is considered as novel biomarker for assessing metastasis and prognosis.

The present study showed 86% of CD44 positive cases of which 46% were showing low expression. CD44 expression showed significant correlation with histological type (intestinal)[p value=0.049], histologic grade (well differentiated)[p value=0.000] and also histological stage (1,2) [p value= 0.000].

Similar studies which were conducted showed the following results.

In a study conducted by Kamran ghaffarzadehgan et al, out of 100 patients studied of gastric carcinoma, 65% of cases were CD44 positive. It was commonly seen in intestinal subtype (p=0.002). Statistical correlation was also significant between CD44 expression and grade of the tumor (p=0.014). It was correlated with poor prognosis with grave survival benefit (p=0.008). No significant correlation was seen with clinical variables.

In another study conducted by Twang et al (43), where 116 cases of gastrectomy specimens of gastric carcinoma were analyzed . IHC expression of stem cell markers like CD44, Musashil and CD133 in gastric tissue samples were analysed. 77% of cases showed CD44 positivity. In this study no difference in expression of CD44 between intestinal and diffuse type was observed. But significant statistical correlation was seen between histological stage and grade of the tumor(p=0.002).

Since CD44 expression has shown a significant association with the stage of the disease and since the stage is a proven prognostic marker in gastric adenocarcinomas, CD44 expression can be used as a prognostic marker to assess the invasiveness and also the metastatic potential of these tumors.

SUMMARY AND CONCLUSION

SUMMARY

In this study, 50 cases of gastric adenocarcinoma were taken randomly, which was diagnosed from the tissue sections obtained from total/subtotal gastrectomy specimens received in our Department of Pathology. The cases with an adequate clinical and investigation data have only been included in the study.

The age group in gastric adenocarcinoma cases were ranging between 23 to 76 years with the peak age of occurrence in the age group of more than 60 years(56%). Males were affected commonly than females(male/female ratio=2.1:1). Most common presenting symptoms were dyspepsia(94%) and epigastric pain(80%) and small proportion of patients also presented with symptoms of vomiting(24%). Majority of cases of gastric carcinoma have significant association with smoking and alcohol. Oesophagogastroduodenoscopy findings of most of the tumors were found to be proliferative growth(82%)

The tumors were graded as well differentiated, moderately differentiated and poorly differentiated and also staged according to TNM system. High number of cases were found to be moderately differentiated tumors and also predominantly found to be in stage 3 at the time of diagnosis.

CD44 expression was studied by immunohistochemistry in all the 50 cases. Scores were given for the intensity of staining as well as for the percentage of tumor cells stained and composite scores for each case has been analyzed. Composite score was calculated by multiplying both the intensity and percentage of tumor cells stained by IHC. Cases were divided into positive and negative for CD44 expression. The CD44 positive cases were further divided by its pattern of positivity as membranous, cytoplasmic or both membranous and cytoplasmic.

By statistical analysis CD44 expression was found to show significant correlation with histological type, grade as well as stage, but showed no significant correlation with age, sex and gross findings such as location and morphology of the tumor in resected specimens.

Three cases of moderately differentiated adenocarcinoma showed negative expression for CD44, which may be explained by advanced stage (stage 4) of the tumor in these cases and can also be attributed to tumor heterogeneity within the same tumor.

Limitation of the study:

As the study has been conducted in smaller sample it needs to be evaluated in larger samples for better evidence of correlation of CD44 versus clinical and histopathological findings.

Scope for future study:

In the future CD44 could further be used for targeted therapy in patients with gastric adenocarcinoma.

BIBLIOGRAPHY

BIBLIOGRAPHY

1. Ferlay J et al. GLOBOCAN 2008, Cancer Incidence and Mortality Worldwide: IARC 2010.

2. Epidemiological review of gastric cancer in India, Rajesh P. Dikshit, Garima Mathur, Sharayu Mhatre, and B. B. Yeole1.Tata Memorial Hospital, Mumbai, India Indian J Med Paediatr Oncol. 2011 Jan-Mar; 32(1): 3–11.

3. The Saudi Journal of Gastroenterology Stomach carcinoma in the Indian subcontinent: A 16-year trend Jijo Velliyappillil Cherian, Ramalingam Sivaraman, Arun Kumar Muthusamy, Jayanthi Venkataraman Department of Gastroenterology, Stanley Medical College, Chennai, India Year : 2007 Volume : 13 Issue : 3 Page : 114-117

4. Mohandas KM, Jagannath P. Epidemiology of digestive tract cancers in India. VI. Projected burden in the new millennium and the need for primary prevention. Indian J Gastroenterol. 2000;19:74–8.

5. Rosai and Ackermann, surgical pathology,10 th edition, pg.no. 629 Lauren P. the two histologic main types of gastric cancer. Diffuse and so called intestinal type carcinoma. Acta Pathol Microbiol Scand 1965, 64:31-4

6. Tamura G. Alterations of tumor suppressor and tumor related genes in the development and progression of gastric cancer. World J Gastroenterol 2006; 12: 192-198

7. Gastric Adenocarcinoma -Review and Considerations for Future Directions Bryan J. Dicken et al Ann Surg. 2005 January; 241(1): 27–39

8. Jothy S. CD44 and its partners in metastasis. Clin ExpMetastasis 2003; 20: 195-201

9. Naor D, Sionov RV, Ish-Shalom D. CD44: structure, function, and association with the malignant process. Adv Cancer Res. 1997;71:241–319.

10. Prognostic significance of CD44s expression in resected non-small cell lung cancer Yoon H Ko et al BMC cancer journal.

11. Heldin P, Karousou E, Bernert B, Porsch H, Nishitsuka K, Skandalis SS. Importance of hyaluronan-CD44 interactions in inflammation and tumorigenesis. Connect Tissue Res. 2008;49:215–218.

12. World J Gastroenterol. 2011 July 21; 17(27): 3220–3228. Relationship between LYVE-1, VEGFR-3 and CD44 gene expressions and lymphatic metastasis in gastric cancer ,Fusun Ozmen et al

13. Screaton GR et al Genomic structure of DNA encoding the lymphocyte homing receptor CD44 reveals at least 12 alternatively spliced exons. Proc. Natl Acad. Sci. USA 1992;89:12160-12164.

14. Pathology International Volume 62, Issue 2, pages 112–119, February 2012 Expression of cancer stem cell markers ALDH1, CD44 and CD133 in primary tumor and lymph node metastasis of gastric cancer, Yuta Wakamatsu et al.

15. Ohgaki H, Matsukura N. Stomach cancer. In: Stewart BW, Kleihues P, eds. World Cancer Report. Lyon: IARC Press, 2003; 197.

16. CD44/CD24 Expression in recurrent gastric cancer:a retrospective analysis Ching-Shya Yong et al.

17. Zbigniew Rudzki, Important CD44, McGill University, Montreal April, 1997

18. WHO classification of tumors, Pathology of genetics of tumors of digestive tract ,Stanley R Hamilton, IARC 2000, pg.no.38

19. Chittukadu kesavan gajalakshmi and viswanathan shanta, Lifestyle and Risk of Stomach Cancer: A Hospital-Based Case-Control Study International Journal of Epidemiology, International Epidemiological Association 1996 , Vol. 25, No. 6. –

20. Hu J, Zhang S, Jia E et al. Diet and cancer of the stomach : Acase-control study in China. Int J Cancer 1988; 41:331-35

21. Tominaga K, A case-control study of stomach cancer and its genesis in relation to alcohol consumption, smoking and familial cancer history. Jpn J Cancer Res 1991; 82: 974-79.

22. Annual Report of Population-Based Cancer Registry of the National Cancer Registry Programme. A Project of the Indian Council of Medical Research, New Delhi, India, 1994.

23. Crawford J. The gastrointestinal tract. 1994 Jerrold R. Turner, The gastrointestinal tract Robbins, 8th edition, pg.no.735.

24. Gore R. Gastrointestinal cancer. Radiol Clin North Am. 1997; 35:295–310.

25. Albert C., Clinical Aspects of Gastric Cancer. Gastrointestinal Cancers: Biology, Diagnosis, and Therapy, Lippincot-Raven; 1995:197–216.

26. Stamenkovic I, Amiot M, Pesando JM, Seed B. A lymphocyte molecule implicated in lymph node homing is a member of the cartilage link protein family.

27. Nagano O, Saya H. Mechanism and biological significance of CD44 cleavage. Cancer Sci. 2004; 95:930–935

28. Sadhna Dhingra et al, Clinicopathologic significance of putative stem cell markers, CD44 and nestin, in gastric adenocarcinoma , Int J Clin Exp Pathol. 2011; 4(8): 733.

29. Cancer res 1996;142:19-50. Adhesion receptors in malignant transformation and dissemination of gastrointestinal tumors. Streit M, Schmidt R, Hilgenfeld RU, Thiel E, Kreuser ED, Germany

30. Pignatelli M, Vessey CI. Adhesion molecules: novel molecular tools in tumor pathology. Hum Pathol 1994;25:849-56.

31. Hynes RO. Integrins: versatility, modulation, and signaling in cell adhesion. Cell 1992;69: 11-25.

32. Rudzki Zt Jothy S. CD44 and the adhesion of neoplastic cells. J Clin Pathol: Mol Pathol 1997.

33. John D. Bancroft, theory and practice of histological techniques, sixth edition

34. Ivan Damjanov, Anderson's pathology, tenth edition.

35. Kamran Ghaffarzadehgan , Expression of cell adhesion molecule CD44 in gastric adenocarcinoma and its prognostic importance, World J Gastroenterol 2008 November 7; 14(41): 6376-6381.

36. Blaser MJ et al, Infection with Helicobacter pylori strains possessing cagA is associated with an increased risk of developing adenocarcinoma of the stomach. Cancer Res. 1995;55: 2111-2115.

37. Putao Cen et al , The Role of CD44 in the Pathogenesis, Diagnosis, and Therapy of Gastric Cancer, Gut Liver. 2011 December; 5(4): 397–405.

38. Ishimoto T et al. CD44 variant regulates redox status in cancer cells by stabilizing the xCT subunit of system xc(-) and thereby promotes tumor growth. Cancer Cell. 2011;19 (3:387–400).

39. Cell, Growth-Inhibitory and Tumor-Suppressive Functions of p53 Depend on Its Repression of CD44 Expression, Samuel Godar et al.

40. Sternberg's Diagnostic surgical pathology, fifth edition, The Stomach, David A. Owen.

41. Heider et al , Differential expression of CD44 splice variants in intestinal and diffuse-type human gastric carcinomas and normal gastric mucosa. Cancer Res 1993; 53: 4197-4203

42. SADHINA DHINGRA et al ,Clinicopathologic significance of putative stem cell markers, CD44 and nestin, in gastric adenocarcinoma .

43. Yamaguchi A, Expression of CD44v6 in advanced gastric cancer and its relationship to hematogenous metastasis and long-term prognosis.J Surg Oncol 2002; 79: 230-235.

44. Saito H, Serum concentration of CD44 variant 6 and its relation to prognosis in patients with gastric carcinoma. Cancer 1998; 83: 1094-1101.

45. Ponta H., Immunohistochemical screening for gastric carcinoma, cancer res. 1993:53.

46. Viala J, Nod 1 responds to peptidoglycan delivered by H.Pylori Cag island, Nat immunology, 1994:755–783

47. Ko JK, Alcohol drinking and cigarette smoking-a partner for gastric ulceration. Zhonghua Yi. 2000; 63

INSTITUTIONAL ETHICAL COMMITTEE, STANLEY MEDICAL COLLEGE, CHENNAI-1

Title of the Work : CD-44 (Cell Adhesion Molecule) Expression in Gastric Adenocarcinoma – Prognostic Importance – A study of 50 cases

Principal Investigator : Dr. J. Priyadharisini

Designation : PG in M.D (Path)

Department : Department of Pathology
Government Stanley Medical College,
Chennai-1

The request for an approval from the Institutional Ethical Committee (IEC) was considered on the IEC meeting held on 06.03.2012 at the Council Hall, Stanley Medical College, Chennai-1 at 2PM

The members of the Committee, the secretary and the Chairman are pleased to approve the proposed work mentioned above, submitted by the principal investigator.

The Principal investigator and their team are directed to adhere to the guidelines given below:

1. You should inform the IEC in case of changes in study procedure, site investigator investigation or guide or any other changes.
2. You should not deviate from the area of the work for which you applied for ethical clearance.
3. You should inform the IEC immediately, in case of any adverse events or serious adverse reaction.
4. You should abide to the rules and regulation of the institution(s).
5. You should complete the work within the specified period and if any extension of time is required, you should apply for permission again and do the work.
6. You should submit the summary of the work to the ethical committee on completion of the work.

29/11/12

MEMBER SECRETARY,
IEC, SMC, CHENNAI

S.NO	BIOPSY NO.	AGE/SEX	SITE	GROSS	HISTOLOGICAL TYPE	GRADE
1	548/11	35/M	Body	Proliferative	Intestinal	Moderately differentiated
2	609/11	44/M	Cardia	Ulcerative	Intestinal	Well differentiated
3	716/11	48/F	Cardia	Proliferative	Intestinal	Moderately differentiated
4	1222/11	70/M	Body	Proliferative	Intestinal	Moderately differentiated
5	1805/11	66/M	Cardia	Proliferative	Intestinal	Poorly differentiated
6	1852/11	62/M	Body	Ulcerative	Diffuse	Poorly differentiated
7	1866/11	74/F	Body	Ulcerative	Diffuse	Poorly differentiated
8	2000/11	72/M	Cardia	Ulcerative	Intestinal	Moderately differentiated
9	2033/11	67/M	Body	Ulcerative	Diffuse	Poorly differentiated
10	2086/11	48/F	Antrum	Proliferative	Intestinal	Moderately differentiated
11	2095/11	52/M	Body	Ulcerative	Diffuse	Poorly differentiated
12	2132/11	44/F	Antrum	Proliferative	Intestinal	Moderately differentiated
13	2247/11	67/M	Body	Proliferative	Intestinal	Moderately differentiated
14	2255/11	49/M	Antrum	Proliferative	Intestinal	Moderately differentiated
15	2298/11	58/F	Body	Ulcerative	Diffuse	Poorly differentiated
16	2311/11	66/M	Antrum	Proliferative	Intestinal	Moderately differentiated
17	2345/11	76/F	Body	Ulcerative	Diffuse	Poorly differentiated
18	2464/11	72/F	Body	Proliferative	Intestinal	Moderately differentiated
19	2474/11	44/M	Body	Proliferative	Intestinal	Moderately differentiated
20	2843/11	72/F	Antrum	Proliferative	Intestinal	Moderately differentiated
21	3043/11	54/M	Antrum	Proliferative	Intestinal	Poorly differentiated
22	3078/11	36/F	Antrum	Proliferative	Intestinal	Moderately differentiated
23	3205/11	67/M	Antrum	Proliferative	Intestinal	Moderately differentiated
24	3206/11	56/F	Antrum	Proliferative	Intestinal	Moderately differentiated
25	3363/11	63/F	Antrum	Proliferative	Intestinal	Moderately differentiated
26	3371/11	52/M	Antrum	Proliferative	Intestinal	Moderately differentiated
27	3523/11	68/M	Cardia	Proliferative	Intestinal	Moderately differentiated
28	3613/11	46/M	Body	Ulcerative	Diffuse	Poorly differentiated
29	3997/11	39/M	Antrum	Proliferative	Intestinal	Moderately differentiated
30	4051/11	62/M	Body	Proliferative	Intestinal	Moderately differentiated
31	4308/11	61/M	Body	Proliferative	Intestinal	Moderately differentiated
32	4313/11	72/F	Body	Proliferative	Intestinal	Poorly differentiated
33	4359/11	76/M	Antrum	Proliferative	Intestinal	Moderately differentiated
34	4386/11	65/M	Antrum	Proliferative	Intestinal	Moderately differentiated
35	4673/11	63/M	Antrum	Proliferative	Intestinal	Moderately differentiated
36	5039/11	38/F	Antrum	Proliferative	Intestinal	Moderately differentiated
37	5101/11	68/F	Antrum	Proliferative	Intestinal	Moderately differentiated
38	5924/11	66/F	Antrum	Proliferative	Intestinal	Moderately differentiated
39	6032/11	71/M	Antrum	Proliferative	Intestinal	Moderately differentiated
40	6423/11	56/M	Antrum	Proliferative	Intestinal	Moderately differentiated
41	37/12	59/M	Body	Proliferative	Intestinal	Moderately differentiated
42	373/12	46/F	Cardia	Proliferative	Intestinal	Well differentiated
43	502/12	48/M	Cardia	Proliferative	Intestinal	Well differentiated
44	530/12	70/M	Body	Proliferative	Intestinal	Poorly differentiated
45	698/12	69/M	Cardia	Proliferative	Intestinal	Well differentiated
46	752/12	67/M	Antrum	Proliferative	Intestinal	Moderately differentiated
47	1338/12	71/M	Cardia	Proliferative	Intestinal	Well differentiated
48	1427/12	59/M	Cardia	Proliferative	Intestinal	Well differentiated
49	1661/12	48/M	Body	Proliferative	Intestinal	Poorly differentiated
50	2222/12	63/M	Cardia	Proliferative	Intestinal	Well differentiated

STAGE	CD44 EXPRESSION
3	Low expression
1	High expression
3	Low expression
3	Low expression
3	Negative
4	Low expression
3	Low expression
3	High expression
3	Low expression
3	Low expression
4	Negative
3	Low expression
1	High expression
3	Low expression
3	Low expression
3	Low expression
3	Low expression
3	High expression
3	Low expression
3	Negative
3	Low expression
3	Low expression
2	Low expression
3	Negative
2	Low expression
2	Low expression
3	Negative
3	Low expression
2	Low expression
2	Low expression
3	High expression
3	Low expression
3	High expression
2	High expression
2	High expression
2	High expression
2	High expression
3	Low expression
2	High expression
1	High expression
1	High expression
1	High expression
1	High expression
4	Negative
1	High expression
2	High expression
1	High expression
1	High expression
4	Negative
1	High expression